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**APPLICATION NUMBER: 60/554,508**

**FILING DATE: *March 19, 2004***

**RELATED PCT APPLICATION NUMBER: *PCT/US05/09417***



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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

EV 324102988 US

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<input checked="" type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
Ginkgolides A and J, and Related Therapeutic Methods and Compositions					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input checked="" type="checkbox"/> Customer Number		28089		Place Customer Number Bar Code Label here	
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ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages		92		<input type="checkbox"/> CD(s) Number	
<input checked="" type="checkbox"/> Drawing(s) Number of Sheets		3		<input checked="" type="checkbox"/> Other (specify)	
<input checked="" type="checkbox"/> Application Data Sheet See 37 CFR 1.76		Return Postcard			
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.				FILING FEE AMOUNT (\$)	
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees					
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number		08-0219		\$80.00	
<input type="checkbox"/> Payment by credit card Form PTO-2038 is attached					
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government					
<input checked="" type="checkbox"/> No					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are:					

Respectfully submitted,

*Jane M. Love*

Date

3/19/04

SIGNATURE

REGISTRATION NO.

42,812

TYPED or PRINTED NAME Jane M. Love, Ph.D.

(if appropriate)

Docket Number

P-00078

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**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

**Additional Page**

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**P-00078**

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Number 2 of 2

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants: Vitolo et al.  
U.S. Serial No. Not Yet Assigned  
Filing Date: Herewith (March 19, 2004)  
For: Ginkgolides A and J, and Related Therapeutic Methods and Compositions

**CERTIFICATE OF EXPRESS MAILING**

I hereby certify that this correspondence and enclosures referenced herein are being deposited with the United States Postal Service as Express Mail under Express Mail Label No. **EV 324102988** **US** postage prepaid in an envelope addressed to: Mail Stop Provisional Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

**TRANSMITTAL LETTER FOR PROVISIONAL APPLICATION**

Sir:

Enclosed are the following for appropriate action:

1. Provisional Application Cover Sheet;
2. 92 pages of Specification, Claims and Abstract;
3. 3 pages of Figures;
4. Application Data Sheet; and
5. Return Postcard.

The Commissioner is authorized to charge the sum of **\$80.00**, which represents the total amount due for filing this provisional patent application to our Deposit Account No. 08-0219. The Commissioner is further authorized to charge any fees under 37 C.F.R. §§ 1.16 and 1.17 associated with the pendency of this application except for an issue fee, or credit any overpayment to our Deposit Account No.08-0219.

Respectfully submitted,



Jane M. Love, Ph.D.  
Registration No. 42,812

Date: 3/19/04

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***Ginkgolides A and J, and Related Therapeutic Methods and Compositions***

**FIELD OF THE INVENTION**

This invention relates to the field of medicine and neurobiology. In particular, this invention relates to methods and compositions for treating or preventing neurological diseases or disorders, as well as neurological damage. The invention further relates to compositions and methods for preventing loss of neuronal long-term potentiation activity and associated functions, as well as for preventing neuronal cell death. The invention further provides compositions and methods for detecting associated neurological and neuroprotective activities.

**1. BACKGROUND OF THE INVENTION**

It is estimated that some 10% of the U.S. population is affected by one form or other of a neurological disease, disorder or trauma. This number is expected to advance to well over 12% of the population by the year 2012 (see [www.brainrestoration.com](http://www.brainrestoration.com)). This corresponds to some 30 million people in the U.S., and billions of individuals worldwide. In the U.S. alone, the associated health care costs are nearly six hundred billion dollars a year. Furthermore, particular types of neurological dysfunction, including Alzheimer's disease, memory loss and depression, are expected to grow acutely in frequency with the aging of the U.S. population.

One historically influential approach to treating neurological disorders has been to look for natural products from plants and other organisms that have therapeutic properties. Traditional Chinese medicine records the use of fruit of the *Ginkgo biloba* fruit (Ginkgo nuts) to treat senility in aging members of the royal court centuries ago (see Drieu, *et al.* (2000) Med. Arom. Plants Ind. Profiles 12: 267). Today, leaf preparations from *G. biloba*, the ginkgo tree, have become one of the best-selling herbal medications worldwide, with Americans spending much of the approximately four billion dollar market in herbal supplements on *G. biloba* extracts.

The *G. biloba* extract Egb 761, one of the first standardized preparations from the ginkgo tree, is obtained from dried green leaves that are extracted with an acetone/water mixture (see McKenna, *et al.* (2001) Altern. Ther. Health Med. 7: 70-86, 88-90). This extract has been standardized to contain 6% terpene trilactones (TTLs) (3.1% ginkgolides and 2.9% bilobalide

(BB)) and 24% flavonoids (Drieu, *et al.* (2000) Med. Arom. Plants Ind. Profiles 12: 453). The flavonoids are predominantly in the form of flavonol-*O*-glycosides, a combination of the phenolic aglycones quercetin, kaempferol, or isorhamnetin, with glucose or rhamnose or both at different positions of the flavonol moiety. The flavonoids may not penetrate the blood-brain barrier (BBB), whereas it appears that the lipophilic character of TTLs renders these compounds permeable to the BBB, and therefore it appears that one or more of the TTLs in *G. biloba* may be at least partially responsible for the observed neurological effects of *G. biloba* extracts.

Egb 761 is the most widely used extract for scientific investigations of the pharmacological effects of *G. biloba*, and several recent reviews have described the effects of the extract on the CNS (MacIennan, *et al.* (2002) Prog. Neurobiol. 67: 235; Darlington *et al.* (2000) Plants Ind. Profiles 12: 331; DeFeudis *et al.* (2000) Curr. Drug Targets 1: 25; and Luo (2001) J. Alzheimer's Dis. 3: 401).

These studies have employed unrefined extracts, such as Egb 761, which contain ginkgolides A, B, C, and J, as well as bilobalide (BB) and a complex mixture of flavonoids and other components. Therefore, these studies do not address which of the known components of Egb 761, or other *G. biloba* extract, are efficacious neurologically, or the molecular basis for their action on the CNS. In addition, although Egb 761 might be beneficial in relieving symptoms of AD and other neurodegenerative diseases and disorders, the reported effects are often small. Furthermore, the postulated effects of Egb 761 in improving memory functions in healthy people are still controversial, despite numerous studies.

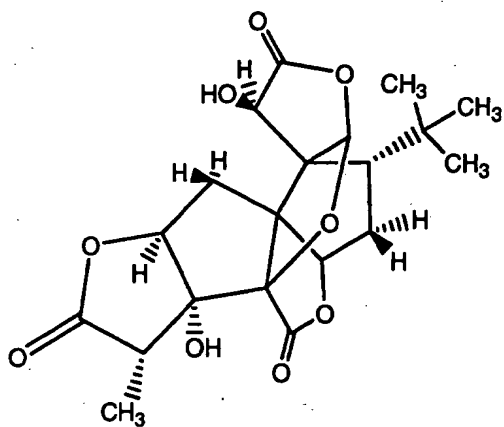
The invention provides an advance in overcoming the problems of weak effectiveness, non-specificity and potential side-effects from the therapeutic use of unrefined *G. biloba* extracts or TTL mixtures.

2. **SUMMARY OF THE INVENTION**

The invention provides, in one aspect, Ginkgolide A and Ginkgolide J compositions and derivatives for the treatment and/or prevention of neurological diseases and disorders, as well as for the improvement of neurological functions, including memory.

In one aspect, the invention provides compositions useful for treating or preventing neurological diseases, disorders or conditions that contain two or more ginkgolides, particularly Ginkgolide A (GA) and Ginkgolide J (GJ), or one or more ginkgolide derivatives, such as or a Ginkgolide A derivative, or a Ginkgolide J derivative.

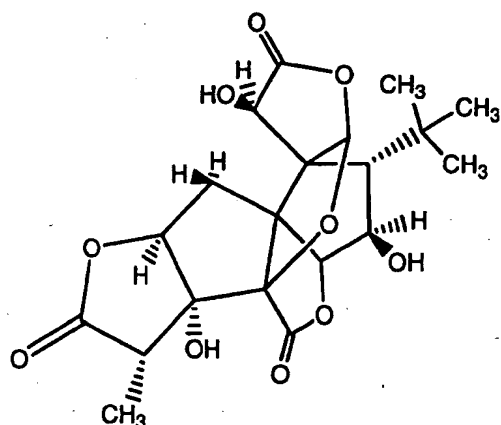
In one aspect, the invention provides a composition that consists essentially of Ginkgolide A (GA) (or derivative thereof) and Ginkgolide J (or derivative thereof). In certain embodiments, the composition includes Ginkgolide A having the formula:



[2].

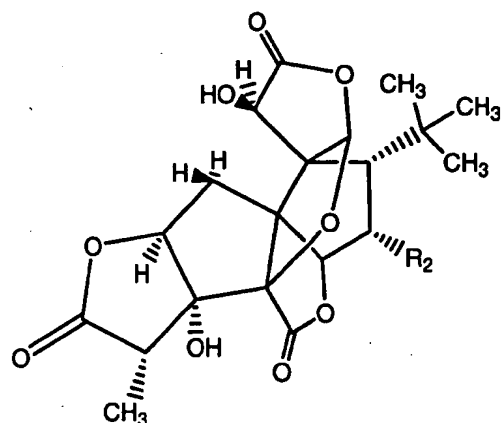
In other embodiments, the composition includes Ginkgolide J having the formula:





[3].

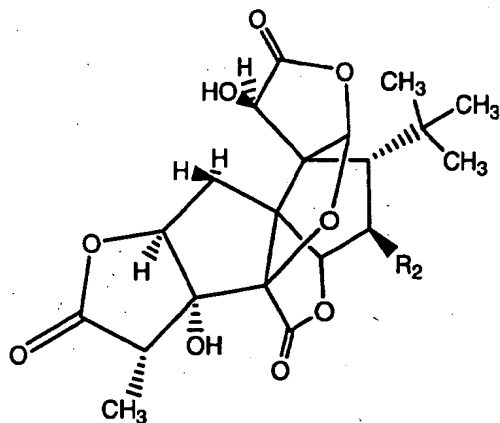
In certain embodiments, the composition includes a Ginkgolide A derivative having the general structure:



[5], where  $R_2$  can be  $NH_2$ , F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-

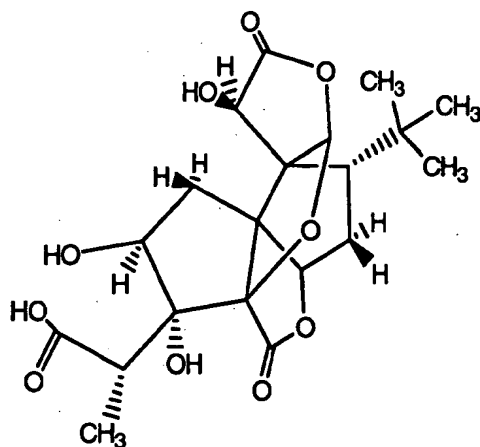
alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

In certain other embodiments, the composition includes a Ginkgolide J derivative having the general structure:



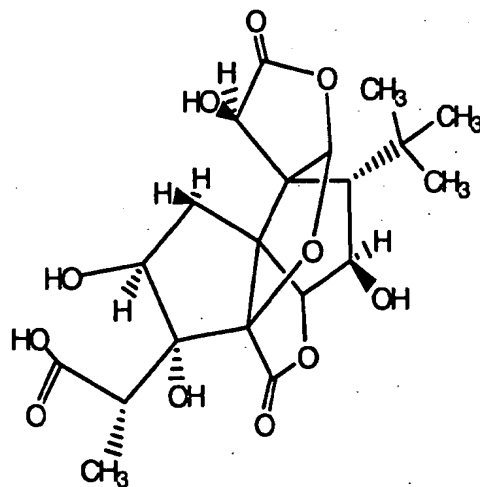
[4], where R<sub>2</sub> can be NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

In still other embodiments, the composition includes a Ginkgolide A derivative that is a monoacid form of Ginkgolide A having the structural formula:



[6].

In other embodiments, the composition includes a Ginkgolide J derivative that is a monoacid form of Ginkgolide AJ having the structural formula:

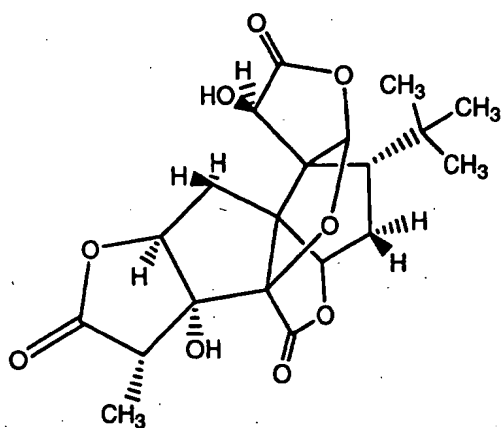


[7].

In still other embodiments, the composition consists essentially of Ginkgolide A and Ginkgolide J, while, in particular embodiments, the composition consists of Ginkgolide A and Ginkgolide J.

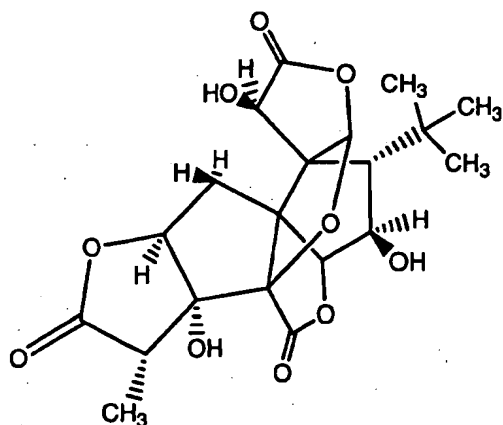
In another aspect, the invention provides compositions consisting essentially of two or more of the compounds Ginkgolide A, a Ginkgolide A derivative, Ginkgolide J and a Ginkgolide J derivative. In this aspect, the invention may consist of Ginkgolide A and/or one or more Ginkgolide A derivatives, in the absence of Ginkgolide J or a Ginkgolide J derivative, or it may consist of Ginkgolide J and/or one or more Ginkgolide J derivatives, in the absence of Ginkgolide A or a Ginkgolide A derivative. For example, in certain embodiments, the invention provides a composition consisting essentially of Ginkgolide A and a Ginkgolide A derivative. In other embodiments, the composition consists essentially of two Ginkgolide A derivatives. In still other embodiments, the invention provides a composition consisting essentially of Ginkgolide J and a Ginkgolide J derivative. In other embodiments, the composition consists essentially of two Ginkgolide J derivatives.

In particular embodiments, the composition includes Ginkgolide A having the formula:



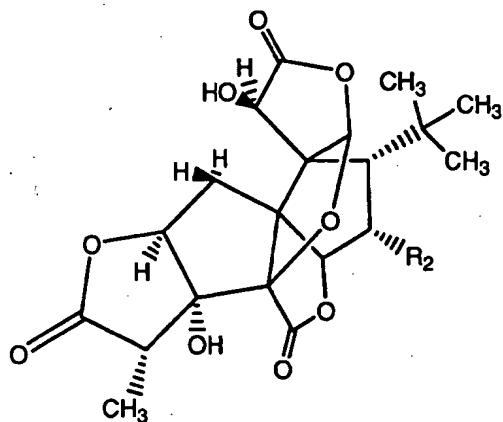
[2].

In additional embodiments, the composition includes Ginkgolide J having the formula:



[3].

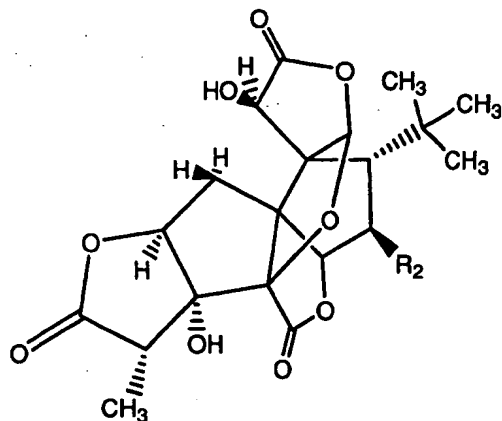
In still other embodiments, the composition includes a Ginkgolide A derivative having the generalized structural formula:



[5], where R<sub>2</sub> can be NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-

alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

In further embodiments, the composition includes a Ginkgolide J derivative having the generalized structural formula:

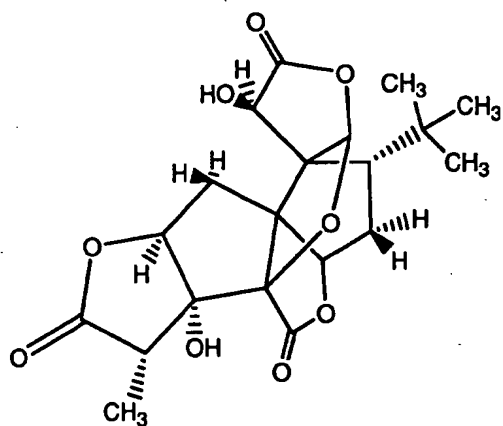


[4], where R<sub>2</sub> can be NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

In another aspect, the invention provides compositions that consist essentially of Ginkgolide A, or a Ginkgolide A derivative, and Ginkgolide J, or a Ginkgolide J derivative, in combination with a pharmaceutical carrier. In particular embodiments, the pharmaceutical carrier is, or includes, a carrier for oral administration, intravenous administration, administration via the respiratory tract, subcutaneous administration, intramuscular administration, rectal administration, topical administration or any combination thereof. In other embodiments, the pharmaceutical carrier is, or includes, a sugar, a starch, cellulose, a cellulose derivative, malt,

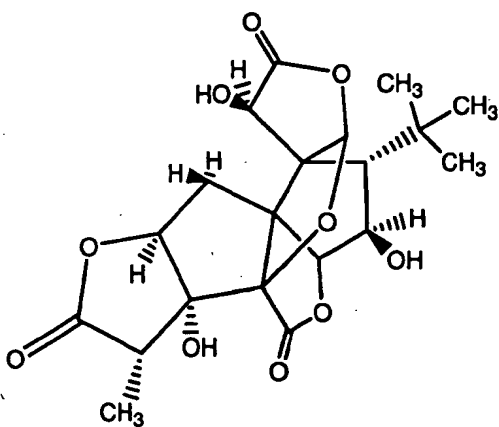
gelatin, a talc, an oil, a glycol, a glycerin, a polyethylene glycol, as ester, an aptamer, magnesium hydroxide, aluminum hydroxide, isotonic saline, Ringer's solution, ethyl alcohol, pyrogen-free water, phosphate buffered saline, sodium lauryl sulfate, magnesium stearate or any combination thereof.

In other embodiments, the pharmaceutical formulation includes a Ginkgolide A having the structural formula:



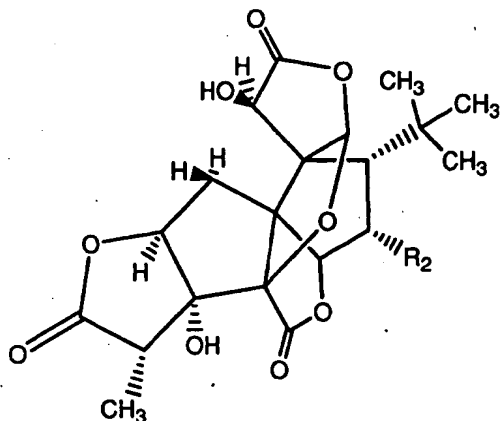
[2].

In further embodiments, the pharmaceutical formulation includes a Ginkgolide J having the structural formula:



[3].

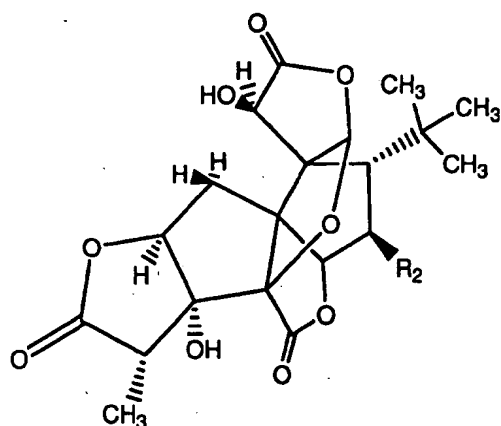
In other embodiments, the composition includes a Ginkgolide A derivative having the generalized structural formula:



[5], where R<sub>2</sub> can be NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

In still other embodiments, the composition includes a Ginkgolide J derivative having the generalized structural formula:





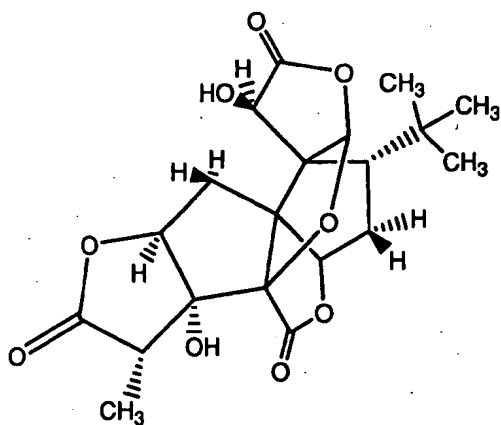
[4], where  $R_2$  can be  $NH_2$ , F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

In further embodiments, the composition consists essentially of Ginkgolide A, Ginkgolide J, and a pharmaceutical carrier, while in particular embodiments, the composition consists of Ginkgolide A, Ginkgolide J, and a pharmaceutical carrier.

In yet another useful aspect, the invention provides a composition consisting essentially of Ginkgolide A (or a Ginkgolide A derivative) and Ginkgolide J (or a Ginkgolide J derivative), in combination with at least one antioxidant. In certain embodiments of this aspect, the composition consists essentially of Ginkgolide A, Ginkgolide J, and at least one antioxidant. In certain embodiments, the included antioxidant is a flavonoid compound. In particular embodiments, the flavonoid is a flavonol glycoside. In particular embodiments, the included flavonol glycoside may be a quercetin, kaempferol, isorhamnetin, or any combination of these compounds. In other embodiments, the included antioxidant is a biflavone. In particular

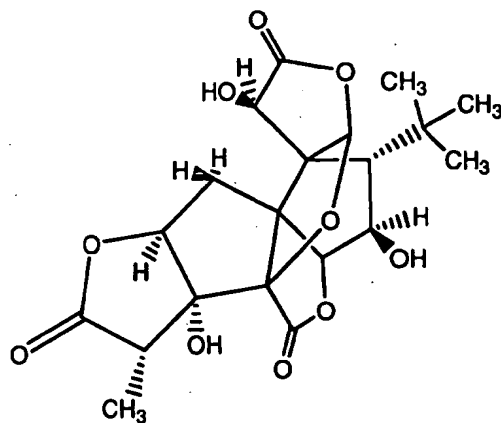
embodiments, the included biflavone may be amentoflavone, ailobetin, ginkgetin, isoginkgetin, sciadopirysin, or any combination of these. In still other embodiments, the composition includes an antioxidant such as vitamin C, vitamin E, N-acetyl-L-cysteine, resveratrol, coenzyme Q, alpha-lipoic acid, lycopene, or any combination thereof.

In still other embodiments, the composition includes a Ginkgolide A having the structural formula:



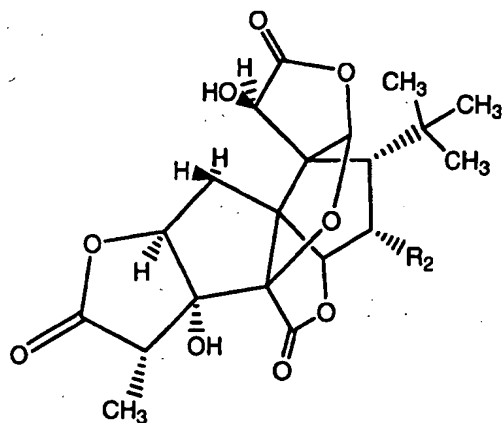
[2].

In further embodiments, the composition includes a Ginkgolide J having the structural formula:



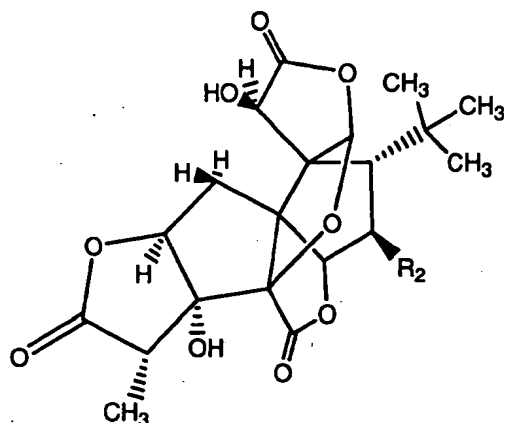
[3].

In still other embodiments, the composition includes a Ginkgolide A derivative having the generalized structural formula:



[5], where  $R_2$  can be  $NH_2$ , F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

In further embodiments, the composition includes a Ginkgolide J derivative having the generalized structural formula:



[4], where  $R_2$  can be  $NH_2$ , F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

In another aspect, the invention provides compositions that consist essentially of four components: Ginkgolide A (or a Ginkgolide A derivative), Ginkgolide J (or a Ginkgolide J derivative), at least one flavonoid, and a pharmaceutical carrier.

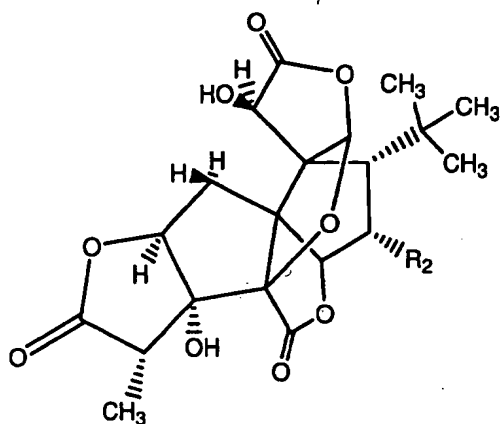
In a further aspect, the invention provides compositions that consist essentially of four components: Ginkgolide A (or a Ginkgolide A derivative), Ginkgolide J (or a Ginkgolide J derivative), at least one antioxidant, and a pharmaceutical carrier.

In still another aspect, the invention provides a composition that consists essentially of three components: Ginkgolide A, Ginkgolide J and bilobalide.

In yet another aspect, the invention provides for the inclusion of a pharmaceutical carrier with any of the aforementioned compositions of the invention. For example, in certain embodiments, any of the aforementioned ginkgolide compositions may include a pharmaceutical carrier that is, or includes, a carrier for oral administration, intravenous administration,

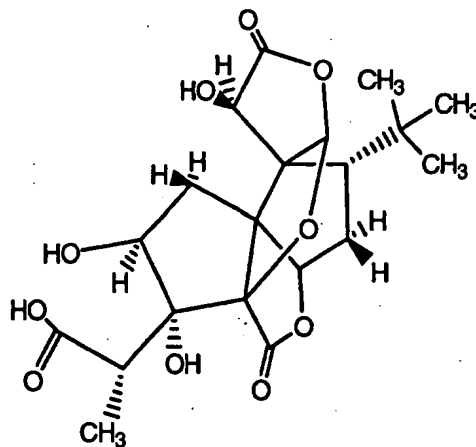
administration via the respiratory tract, subcutaneous administration, intramuscular administration, rectal administration, or topical administration. In further embodiments, the pharmaceutical carrier may be a sugar, a starch, cellulose, a cellulose derivative, malt, gelatin, a talc, an oil, a glycol, a glycerin, a polyethylene glycol, as ester, an aptamer, magnesium hydroxide, aluminum hydroxide, isotonic saline, Ringer's solution, ethyl alcohol, pyrogen-free water, phosphate buffered saline, sodium lauryl sulfate, and magnesium stearate.

In yet another important aspect, the invention provides compositions that include a Ginkgolide A derivative or a Ginkgolide J derivative. In certain embodiments, a Ginkgolide A derivative is included. In other embodiments, a Ginkgolide J derivative is included. For example, in some embodiments, the composition includes a Ginkgolide A derivative having the generalized structural formula:



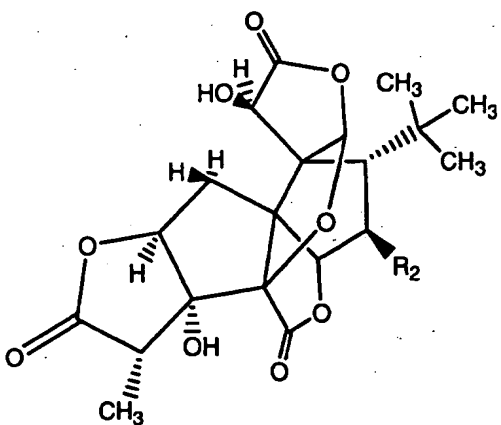
[5], where  $R_2$  can be  $NH_2$ , F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted. In other

embodiments, the composition includes a monoacid Ginkgolide A derivative having the structural formula:



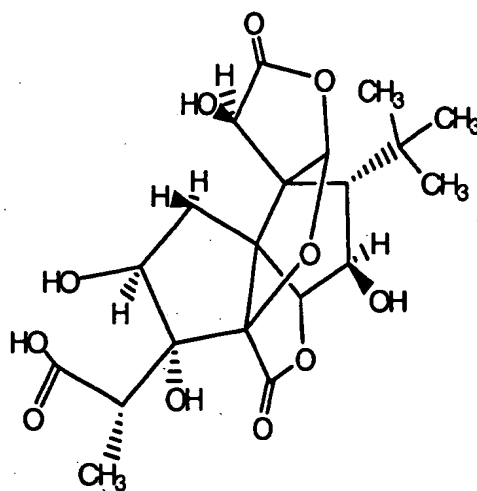
[6].

In still other embodiments, the other embodiments, the composition includes a Ginkgolide J derivative having the generalized structural formula:



[4], where R<sub>2</sub> can be NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an

unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted. In further embodiments, the composition includes a monoacid Ginkgolide J derivative having structural the formula:



[7].

In a particularly significant aspect of the invention, the aforementioned compositions are useful for treating a neurodegenerative disease or disorder. In certain aspects, the aforementioned compositions are useful for treating a memory disorder. In further aspects, the aforementioned compositions are useful for improving memory.

In certain particularly useful embodiments, the aforementioned compositions are useful to treat Alzheimer's disease. In particular embodiments, the aforementioned compositions protect against neuronal cell death or long term potentiation impairment by beta amyloid protein.

In another aspect, the invention provides methods for treating a neurological or neurodegenerative disease or disorder in a mammal by administering any of the aforementioned compositions. In a further aspect, the invention provides methods for treating a memory disorder in a mammal by administering any of the aforementioned compositions.

In still another aspect, the invention provides methods for memory in a mammal by administering by administering any of the aforementioned compositions.

In a particularly useful aspect, the invention provides a method of treating depression in a mammal by administering any of the aforementioned compositions.

In particular embodiments, the methods of the invention provide a means of treating Alzheimer's disease, a neurological or neurodegenerative disease. In certain other embodiments, the methods of the invention provide a means of treating trisomy 21 (Down's syndrome).

In further embodiments, the method of the invention may be used to treat any neurological or neurodegenerative disease or disorder such as, but not limited to, multiple sclerosis, amyotrophic lateral sclerosis, Alpers' disease, corticobasal ganglionic degeneration, multiple system atrophy, motor neuron disease, olivopontocerebellar atrophy, Parkinson's disease, prion disease, Rett syndrome, tuberous sclerosis, Shy-Drager syndrome, Huntington's disease, senile dementia, epileptic dementia, presenile dementia, post-traumatic dementia, vascular dementia and post stroke dementia, alcoholism, meningitis, neonatal hypoxia, stroke, global cerebral ischemia, or any combination thereof.

In particular embodiments, the method includes the administration of composition that promotes neurogenesis in the treated mammal. In certain particularly useful embodiments, the composition is administered in an amount effective to protect against neuronal cell death or long term potentiation impairment by beta amyloid protein.

In further embodiments, the invention provides methods for treating neurological damage in a mammal by administering any of the aforementioned compositions in an amount effective to cause neurite out growth. In particularly useful embodiments, the neurological damage treated is a spinal chord injury.

In various embodiments, the methods of the invention provide for different routes of administration of the therapeutic compositions of the invention. For example administration may be oral, intravenous, subcutaneous, intramuscular, rectal, topical, or through the respiratory tract.

In still another particularly useful aspect, the invention provides a method for protecting a neuron against neuronal cell death, and/or long term potentiation impairment, by beta amyloid protein. In this aspect, the method of the invention includes the step of contacting the neuron with Ginkgolide A.



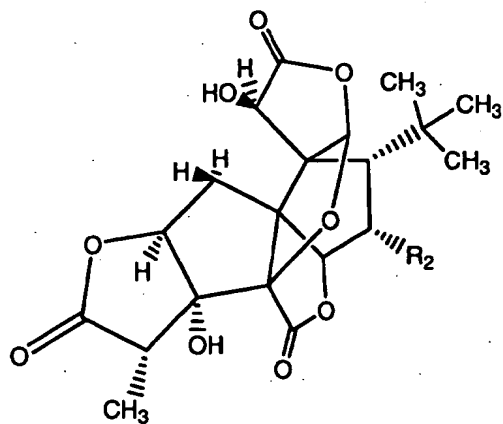
In another particularly useful aspect, the invention provides a method for protecting a neuron against neuronal cell death, and/or long term potentiation impairment, by beta amyloid protein. In this aspect, the method of the invention includes the step of contacting the neuron with Ginkgolide J.

In still another particularly useful aspect, the invention provides a method for protecting a neuron against neuronal cell death, and/or long term potentiation impairment, by beta amyloid protein. In this aspect, the method of the invention includes the step of contacting the neuron with Ginkgolide A and Ginkgolide J.

In certain particularly useful embodiments of these methods, the neuron protected is contacted with a composition consisting essentially of Ginkgolide A and Ginkgolide J.

In a further aspect, the invention provides a method for stimulating axonal outgrowth of a neuron by contacting the neuron with Ginkgolide A.

In one aspect, the invention provides a method for stimulating axonal outgrowth of a neuron by contacting the neuron with a Ginkgolide A derivative having the formula:

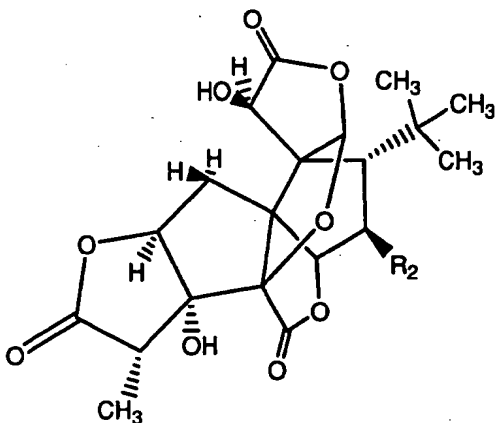


[5], where R<sub>2</sub> can be NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an

unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted. In some embodiments, the method further includes the step of contacting the neuron with Ginkgolide J or a Ginkgolide J derivative.

In a further aspect, the invention provides a method for stimulating axonal outgrowth of a neuron by contacting the neuron with Ginkgolide J.

In one aspect, the invention provides a method for stimulating axonal outgrowth of a neuron by contacting the neuron with a Ginkgolide J derivative having the formula:

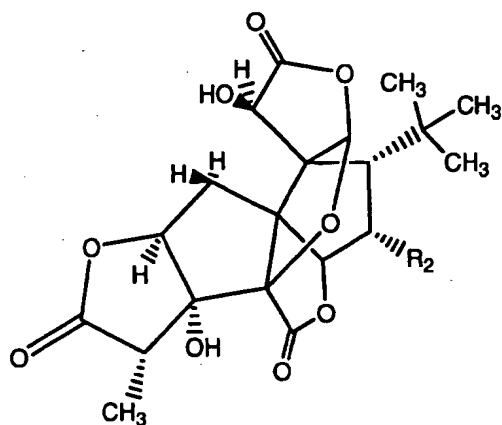


[4], where R<sub>2</sub> can be NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted. In some embodiments, the method further includes the step of contacting the neuron with Ginkgolide A or a Ginkgolide A derivative.

In yet another aspect, the invention provides a method of treating a neurological or neurodegenerative disease or disorder in a mammal by administering an enriched *Ginkgo biloba* extract comprising at least about 60% terpene trilactones.

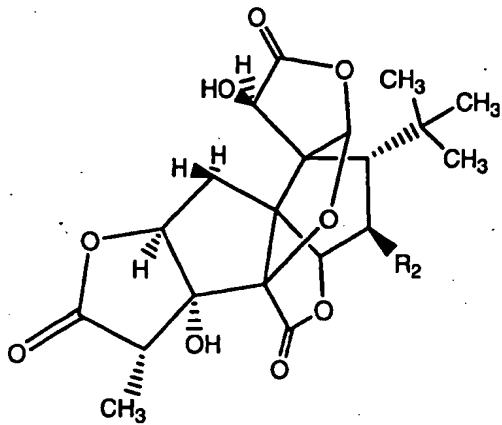
In another aspect, the invention provides a method of protecting a neuron against neuronal cell death, or long term potentiation impairment, by beta amyloid protein by contacting the neuron with an enriched *Ginkgo biloba* extract comprising at least about 60% terpene trilactones.

In a further aspect, the invention provides a method of identifying a receptor that binds a compound that protects against neuronal cell death or long term potentiation impairment by beta amyloid protein by administering a Ginkgolide A derivative having generalized structural formula:



[5], where R<sub>2</sub> can be a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety. In certain embodiments, the method is diagnostic of Alzheimer's disease.

In still another aspect, invention provides a method of identifying a receptor that binds a compound that protects against neuronal cell death or long term potentiation impairment by beta amyloid protein by administering a Ginkgolide J derivative having generalized structural formula:



[4], where R<sub>2</sub> can be a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety. In certain embodiments, the method is diagnostic of Alzheimer's disease.

3. **BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 is a graphical representation showing that a terpene trilactone 70% enriched preparation (P8A) reverses the inhibition of tetanus-induced neuronal long-term potentiation by amyloid-beta protein ( $\beta$ A).

Figure 2A is a graphical representation showing that Ginkgolide A (GA) and Ginkgolide J (GJ) both reverse the inhibition by amyloid-beta protein ( $\beta$ A) of tetanus-induced neuronal long-term potentiation in the CA1 region of hippocampal slices.

Figure 2B is a graphical representation showing Ginkgolide B (GB) and Ginkgolide C (GC), as well as bilobalide (BB) fail to reverse the inhibition of tetanus-induced neuronal long-term potentiation by amyloid-beta protein ( $\beta$ A).

Figure 3 is a graphical representation showing the results of a neuroprotection assay which demonstrates that both a terpene trilactone enriched preparation (P8A) and Ginkgolide J alone protect hippocampal neurons from amyloid-beta protein ( $\beta$ A)-induced cell death.

**4. DETAILED DESCRIPTION OF THE INVENTION**

The patent and scientific literature referred to herein establishes knowledge that is available to those of skill in the art. The issued U.S. patents, allowed applications, published foreign applications, and scientific literature references, including GenBank database sequences, that are cited herein (including those in the Background of the Invention and all other sections) are hereby incorporated by reference to the same extent as if each was specifically and individually indicated to be incorporated by reference for all purposes.

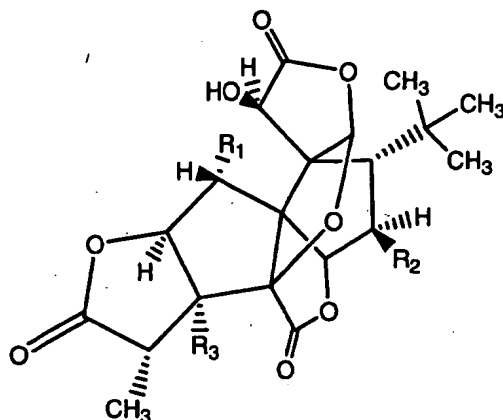
**4.1. General**

The potentially potent and specific therapeutic benefits of one or more individual components remains untapped. Indeed, if only a subset of the TTLs or other components are effective in treating or preventing neurological damage, then the potency of the treatment would be greatly increased by use of those individual component(s), which would avoid the potential for anti-therapeutic side-effects effects that might be caused by the remaining components.

The invention is based, in part, upon the finding that particular Ginkgolide species are potent molecular protectors against neurodegenerative processes including beta amyloid-induced neuronal cell death and impairment of long-term potentiation (LTP). For example, Ginkgolide A (GA) and Ginkgolide J (GJ) are potent in alleviating the inhibition of neuronal long-term potentiation caused by the neurodegenerative agent beta-amyloid protein ( $\beta$ A). Furthermore, Ginkgolide J is potent inhibitor of  $\beta$ A-induced neuronal cell death.

In general, the invention provides compositions for treating or preventing neurological diseases, disorders or conditions, and/or improving memory, as well as methods for their use. Certain of the ginkgolide compositions of the invention contain two or more ginkgolides or ginkgolide derivatives, particularly Ginkgolide A (GA) and/or a Ginkgolide A derivative, and Ginkgolide J and/or a Ginkgolide J derivative.

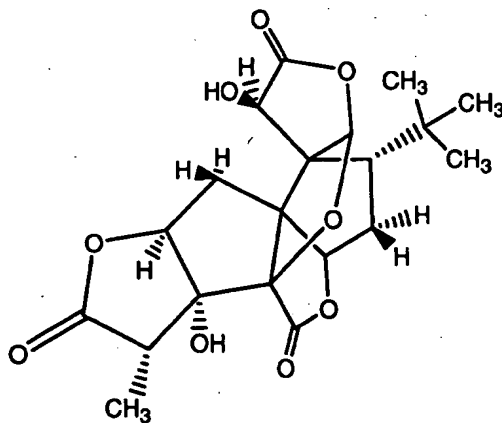
There are five ginkgolides (Ginkgolide A (GA), Ginkgolide B (GB), Ginkgolide C (GC), Ginkgolide J (GJ) and Ginkgolide M (GM)). The generalized structure of the ginkgolides is as follows in formula 1:



[1]

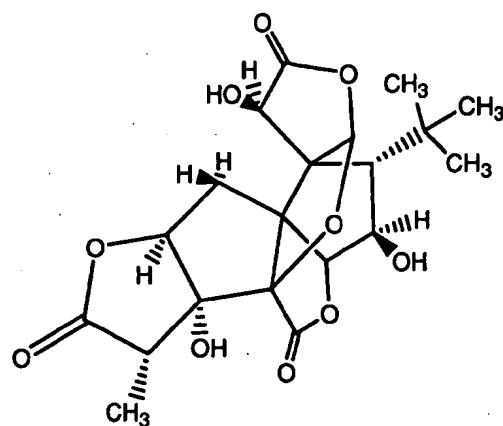
wherein, the structure at each of the variable "R" groups, R1, R2 and R3, varies uniquely in each ginkgolide species as follows: Ginkgolide A (R1 = H, R2 = H, R3 = OH); Ginkgolide B (R1 = OH, R2 = H, R3 = OH); Ginkgolide C (R1 = OH, R2 = OH, R3 = OH); Ginkgolide J (R1 = H, R2 = OH, R3 = OH); and Ginkgolide M (R1 = OH, R2 = OH, R3 = H).

Accordingly, Ginkgolide A has the structure as follows in formula 2:



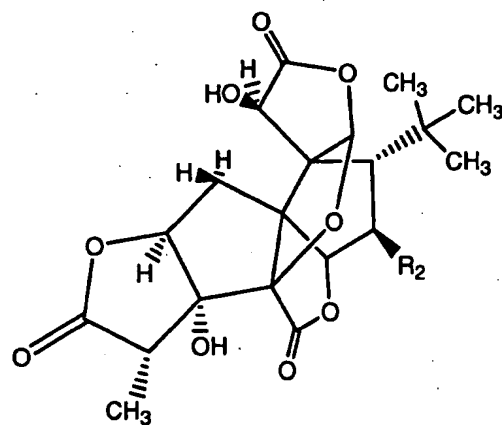
[2]

Ginkgolide J has the structure as follows in formula 3:



[3]

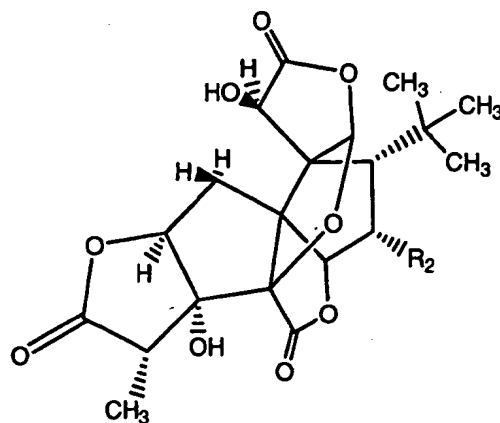
Accordingly, Ginkgolide J derivatives of the invention include those having the structure in formula 4:



[4]

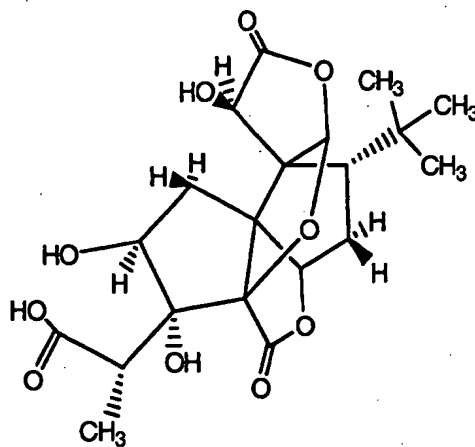
While Ginkgolide A derivatives of the invention include those having the structure in formula 5:





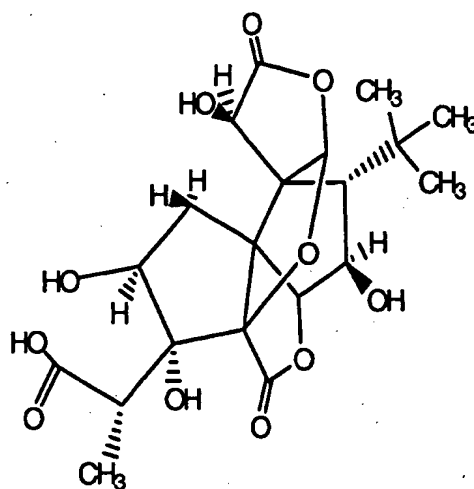
[5]

Furthermore, the invention provides an open-ringed monoacid form of Ginkgolides A and of Ginkgolide J, and derivatives thereof, obtained by the controlled hydrolysis of one of the lactone rings of the ginkgolide structure. The monoacid derivative of Ginkgolide A has the following structure (formula 6):



[6]

While the monoacid derivative of Ginkgolide J has the following structure (formula 7):



[7]

In each case, the "R2" of the ginkgolide derivative may be NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

#### 4.2 Definitions

The term "about" is used herein to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 20%.

The term "β-amyloid protein" or "β-amyloid peptide," refers to a glycoprotein associated with Alzheimer's disease and derived from a precursor, named A4, which is capable of being made in several forms through alternative splicing. The full-length human A4 protein is 770 amino acids long (86.85 kD) (see GenBank Accession Nos. P05067 and QRHUA4), while the β-amyloid protein is shorter carboxy-terminal fragment of the full-length (see GenBank Accession No. 1IYTA (Chain A, Solution Structure Of The Alzheimer's Disease Amyloid Beta- Peptide (1-42)) and, *e.g.*, GenBank Accession No. AAA51726).

The term "antioxidant" refers to any substance, often an organic compound, that opposes oxidation or inhibits reactions brought about by dioxygen or peroxides. Usually the antioxidant is effective because it can itself be more easily oxidized than the substance protected. The term includes components that can trap free radicals, such as α-tocopherol (vitamin E), thereby breaking the chain reaction that normally leads to extensive biological damage. As used herein, the term "antioxidant" includes biological molecules that serve as natural free-radical scavengers intracellularly including ascorbate (vitamin C), glutathione, N-acetyl-L-cysteine and NAD<sup>+</sup>.

"Antioxidant" further includes natural and synthetic compounds that protect labile compounds during storage or incubation (*e.g.*, di-tert-butyl-p-cresol, and quinhydrone). The term includes plant flavonoids, such as the flavonol glycosides quercetin, kaempferol and isorhamnetin, as well as biflavones, such as amentoflavone, ailobetin, ginkgetin, isoginkgetin and sciadopirysin. Still other antioxidants included within the scope of the term are resveratrol, coenzyme Q, α-lipoic acid, and lycopene.

An "effective amount," when used in connection with a composition for administration to a subject, is an amount effective for treating or preventing a specified neurological disease, disorder or condition, or for improving memory, including improving memory in a normal individual, or for inhibiting a specific neurodegenerative process such as neuronal cell death or neuronal long-term potentiation inhibition caused by beta amyloid protein.

The term "flavonoid," as used herein, includes any flavone, isoflavone, or neoflavone, or any of their derivatives, including especially flavone glycosides, biflavones, and bioflavonoids. Flavonoids include a large group of water-soluble phenolic derivatives, often brightly colored, and are typically found in the vacuoles of plant cells. They include the flavone glycosides, with the structure of their aglycon moieties based on the flavan skeleton, and are classified according to the oxidation state of their pyran ring (some 10-12 classes are well known).

The term "ginkgolide," refers generally to a family of bioactive terpenes that were first isolated from the root bark and leaves of the *Ginkgo biloba* plant. The generalized structure of ginkgolide [1] varies at three carbon centers distinguished by a particular combination of side groups (R1, R2, and R3) unique to each form of Ginkgolide (*i.e.*, A, B, C and J). The chemical identity of the side groups in each Ginkgolide is: Ginkgolide A (R1 = H, R2 = H, R3 = OH); Ginkgolide B (R1 = OH, R2 = H, R3 = OH); Ginkgolide C (R1 = OH, R2 = OH, R3 = OH); Ginkgolide J (R1 = H, R2 = OH, R3 = OH); and Ginkgolide M (R1 = OH, R2 = OH, R3 = H).

The term "ginkgolide A derivative," refers to ginkgolides having the structural formula [5], having a non-H R2 substituent present in the S chiral form, wherein the S chiral form is based upon an assignment of R2 as the highest priority substituent regardless of its actual priority over the other two substituents at this chiral center. In addition, the term "ginkgolide A derivative" is meant to include the monoacid derivative of Ginkgolide A having the structural formula [6].

The term "ginkgolide J derivative," refers to ginkgolides having the structural formula [4], having a non-H R2 substituent present in the R chiral form, wherein the R chiral form is based upon an assignment of R2 as the highest priority substituent, regardless of its actual priority over the other two substituents at this chiral center. In addition, the term "ginkgolide J derivative" is meant to include the monoacid derivative of Ginkgolide J having the structural formula [7].

The term "glycoside," refers to any of various kinds of molecules (including flavonoids, such as the flavonol glycosides or flavonol-O-glycoside), which have a sugar group covalently attached to the molecule. The corresponding molecule without the sugar (*e.g.*, flavonol) is known as an "aglycone" when it is not bonded to a sugar molecule. The term "glycoside" as used herein, includes sugars forming an  $\alpha$ - or  $\beta$ -glycosidic linkage. Representative examples of glycosides include, but are not limited to ribose, deoxyribose, fructose, galactose, glucuronic acid and glucose.

The term "isolated," used in reference to a compound, means that the compound is substantially free of other compounds, although other compounds may be present in trace amounts or in amounts insufficient to substantially alter the chemical or biological properties of the "isolated" compound. The term "isolated," when applied to a composition or mixture of

compounds, means that the composition is substantially free of compounds, other than those defining the composition.

The term "isomer" refers to any of two or more compounds that have identical molecular formulas but differ in the nature or arrangement of their atoms. The term "stereoisomers" refers to isomers that differ only in the three-dimensional arrangement of their atoms in space. Two stereoisomers typically differ in the arrangement of their atoms in space at one or more chiral centers. A chiral chemical compound, *i.e.*, one that possesses chirality, is one that cannot be superimposed on its mirror image, either as a result of simple reflection or after rotation and reflection.

As used herein, the term "long-term potentiation" (or "LTP") refers to any long-lasting enhancement of the effectiveness of synaptic transmission that follows certain types of conditioning stimulation. LTP is a form of long-term synaptic plasticity that is a critical cellular basis of learning and memory storage. For example, selective inhibition of NMDA receptor channels has been shown to block LTP, and to block spatial learning.

The term "substituted," when referring to a chemical group that is part of a chemical compound, refers to the replacement of one or more hydrogen atoms of the chemical group with another atom or chemical group such as a halo atom, an amino group, an hydroxyl, or a carboxyl group.

A "subject" includes any mammal, *e.g.*, a human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, or non-human primate, such as a monkey, chimpanzee, or baboon.

#### 4.3 Ginkgolides A and J and Related Derivatives

The invention provides isolated ginkgolides, particularly Ginkgolides A and J (GA and GJ), as well as derivatives of these ginkgolides. Ginkgolides A and J may be obtained from a natural source, such as an unenriched *Ginkgo biloba* extract (such as Egb761), or an enriched terpene trilactone (TTL) extract (see *e.g.*, U.S. Patent No. 6,590,109), or may be chemically synthesized (*e.g.*, by *de novo* chemical synthesis as has been described by E.J. Corey (see Corey and Cheng (1995) The Logic of Chemical Synthesis, John Wiley & Sons, New York, pp. 89-91, 221-226). The ginkgolides and ginkgolide derivatives of the invention may be obtained by chemical synthesis using such known chemical methods, and/or routine modifications of such methods.

Where the ginkgolides are derived from a natural source, such as *Ginkgo biloba* plant parts, they will generally be most efficiently isolated in a relatively pure state by first isolating an enriched mixture of plant terpene trilactones. The first step in obtaining such an enriched TTL preparation may be extraction from the leaves. In most cases, various water-containing solvent systems such as water/acetone or water/methanol are used. Apolar constituents are excluded, whereas all of the TTLs are collected, including GB, which, like the other ginkgolides, is only scarcely soluble in water. Basic extractions should be avoided owing to the instability of BB (bilobalide) in solutions with pH > 7. Recently, an improved extraction of TTLs that takes advantage of their stability under a variety of conditions, such as oxidation and heat, was developed (see Lichtblau *et al.* (2002) *J. Nat. Prod.* 65: 1501). During this process, the leaves are boiled for 1 hour in dilute hydrogen peroxide, followed by extraction with ethyl acetate to generate an off-white powder with a TTL content of 60-70%. The treatment with hydrogen peroxide removes the constituents that lead to extensive emulsions in the extraction steps, thus greatly shortening the process.

Preferred detailed methods for preparing enriched TTL extracts have been described (see U.S. Patent No. 6,590,109). In brief, the method involves isolating and concentrating terpene trilactones from pharmaceutical powder produced by extraction of the leaves of *Ginkgo biloba*, for example Egb761, and involves the following procedures: (A) The dried leaves or the pharmaceutical powder are suspended in water, or in an aqueous solution containing hydrogen peroxide at a concentration range of 0.1-30%, or in an aqueous solution containing hydrogen peroxide at a concentration range of 0.1-30% and additional 0.1-15% percent of mineral acids like acetic, hydrochloric, nitric, phosphoric or sulfuric acid; (B) the resulting suspension is stirred only, or stirred and heated, or stirred and boiled for a period of time between 5 min to 5 hours; (C) if necessary, the pH is adjusted after this treatment to a range of pH 4-6 with alkali hydroxide aqueous solutions such as sodium hydroxide or potassium hydroxide; (D) the suspension of (B) or (C) is extracted with lower acetates, lower ketones, lower ether, lower alcohols or benzenes, by separating the organic layer from the aqueous solution; (E) the resulting organic layer is washed with an aqueous based solution, such as ammonium chloride, sodium carbonate, sodium bicarbonate, potassium carbonate or with alkali hydroxide aqueous solutions such as sodium hydroxide or potassium hydroxide, or with an aqueous solutions such as sodium thiosulfate, sodium sulfite and sodium hydrosulfide or destroy the excess of hydrogen peroxide with metal

dust or on metal based catalysts, or with both such solutions; the organic layer is washed water and/or with an aqueous solution of sodium chloride; (G) the remaining organic layer is separated, dehydrated and dried, so as to remove solvent and obtain an extract which contains more than 50% terpene trilactones; and (H) the extract from step (G) is recrystallized with a mixture of water/methanol or any other acceptable solvent system to obtain the ginkgolides in high purity.

Next, to obtain individual isolated ginkgolide species such as GA and/or GJ, the individual ginkgolides and bilobalide (BB) are separated. The terpene trilactones can be isolated by further processing, for example by reversed phase chromatography, to reduce any unwanted ginkgolic acids. For example, chromatography can be performed with silica gel.

Bilobalide is relatively easily separated from the ginkgolides by using standard column chromatography. The separation of the individual ginkgolides requires further methodology. The ginkgolides differ only by one or two hydroxyl groups, but in some cases these hydroxyl groups are involved in hydrogen bonding, and therefore do not significantly alter the overall polarity of the molecule. Accordingly, a simple improvement in the separation has been described by van Beek and Lelyveld, in which the hydrogen bonds are disrupted by using silica gel impregnated with sodium acetate. This technique is coupled with preparative-scale medium-pressure liquid chromatography (MPLC) (van Beek *et al.* (1997) J. Nat. Prod. 60: 735) and TLC detection of TTLs (van Beek *et al.* (1993) Phytochem. Anal. 4: 109). In the purification step, the labile nature of BB should be considered, as it degrades on alumina columns (van Beek *et al.* (1996) Phytochem. Anal. 7:185). Quantitative <sup>1</sup>H NMR spectroscopic analysis was used as a convenient method to determine the amount of TTLs in various preparations; (Choi *et al.* (2003) Chem. Pharm. Bull. 51: 158) since the signals for the 12-H of TTLs (Rounestand *et al.* (1989) Tetrahedron 45: 1975) are distinct and well-separated, integration intensities of these signals are then compared to that of maleic acid (MA) (van Beek *et al.* (1993) Phytochem. Anal. 4:261). Another useful method, albeit most practical for rapid analytical, rather than preparative, procedures is a method using HPLC on a Lichrosorb C18 (10  $\mu$ m) analytical column with methanol-water-orthophosphoric acid (25:75:0.1, v/v) as eluent and refractive index (RI) detection (see Wang *et al.* (2000) Chin. J. Chrom. 18: 394-97).

TTLs lack common chromophores and therefore UV detection is not ideal. Accordingly, other detection methods, such as refractive index (RI), evaporation light-scattering detection (ELSC), and MS have been utilized. The first reported separation and quantification of TTLs,

however, involved conventional HPLC/UV detection (see Teng *et al.* (1988) J. Chromatogr. 267: 431). RI detection has also been described (van Beek *et al.* (1991) J. Chromatogr. 543: 375). Although UV detection is often preferred as it demonstrates better sensitivity than RI detection, the selectivity is superior when using RI. Several methods have been described using liquid chromatography/ mass spectrophotometry (LC/MS) to separate and quantify TTL content in various *G. biloba* extracts, as well as in plasma after intake of *G. biloba* extract. The major difference in the detection systems lies in the MS procedures used, which could be electrospray ionization (ESI) (Mauri *et al.* (1999) J. Mass Spectrom. 34: 1361) or atmospheric-pressure chemical ionization (APCI) (Mauri *et al.* (2001) Rapid Commun. Mass Spectrom. 15: 929; Jensen *et al.* (2002) Phytochem. Anal. 13: 31). Furthermore, LC/MS (ESI) has been used for analysis of commercial *G. biloba* products in which large variations in the composition and concentration of TTLs were observed (Li *et al.* (2002) Analyst 127: 641). In addition, as an alternative to MS, ELSD was also successfully applied to quantify TTLs in *G. biloba* extracts (Camponovo *et al.* (1995) Phytochem. Anal. 6: 141; Ganzera *et al.* (2001) Chem. Pharm. Bull. 49: 1170).

In addition to purification from a natural source, the ginkgolides and related compounds of the invention may be chemically synthesized. Corey and co-workers have described the total syntheses of both GA (Corey and Ghosh (1988) Tetrahedron Lett. 29: 3205) and GB (Corey *et al.* (1988) J. Am. Chem. Soc. 110: 649). The classic total synthesis of GB has been extensively reviewed (Nicolaou *et al.* (1996) Classics in Total Synthesis VCH, Weinheim p. 451) and only the detailed methodology is well known in the art. Briefly, the key transformations include the following.

A spirocyclic ring system composed of rings B and C of GB is constructed from 2-(2,2-dimethoxyethyl)-cyclopent-2-enone by a 1,4-addition of a tert-butyl cuprate reagent, followed by a Mukaiyama condensation after treatment with 1,3,5-trioxane and titanium chloride to give an intermediate using the tert-butyl moiety as a directing group for the ring closure. A sequence of steps involving a palladium-mediated Sonogashira coupling, an intramolecular ketene-olefin [2+2] cycloaddition, and a Baeyer-Villiger oxidation furnished an intermediate, which contains four of the six rings in GB. For the formation of the tetrahydrofuran moiety, ring D, the reactivity of ring C was modified by introduction of dithiane, and intramolecular etherification provided this intermediate in three steps. Oxidation of ring A and elimination in ring C furnished an intermediate, which was selectively oxidized to provide an epoxyketone in ring A.



This was followed by an intermolecular aldol condensation and lactonization with concomitant opening of the oxirane to give an intermediate with all six rings of GB in place. The total synthesis was completed by dihydroxylation of the double bond in ring C and oxidation to GB. Later, Corey and co-workers suggested an enantioselective route to GB by synthesis of a key intermediate (Corey *et al.* (1988) Tetrahedron Lett. 29: 3201) and various ginkgolide derivatives were also synthesized (Corey *et al.* (1990) Tetrahedron Lett. 31: 3995; Corey *et al.* (1991) Tetrahedron Lett. 32: 4623).

Another method for synthesis of GB has been reported by Crimmins and coworkers (Crimmins *et al.* (1989) Tetrahedron Lett. 30:5997). This method utilizes an intramolecular cycloaddition reaction that efficiently generated the multiple-ring skeleton of ginkgolides. The synthesis started from a furan enone which is very similar to an intermediate created in the synthesis of bilobalide. This furan enone undergoes intramolecular photocycloaddition upon irradiation at > 350 nm to give a polycyclic structure analogous to rings A, B, and C of GB, as a single diastereomer. Inversion of the MOM-protected (methoxymethyl-protected) alcohol and formation of a bridging lactone, is followed by ring expansion of the cyclobutane into a tetrahydrofuran system in six steps to give the desired structure, which is comparable to the analogous Corey intermediate, but lacks crucial functional groups and the tert-butyl group (Crimmins *et al.* (1989) Tetrahedron Lett. 30:5997). In yet another method for the total synthesis of GB, a photocycloaddition is used as a key transformation (Crimmins *et al.* (1999) J. Am. Chem. Soc. 121: 10249).

Methods for synthesizing ginkgolide derivatives, particularly Ginkgolide A and Ginkgolide J derivatives, have been described and/or may be elucidated using routine skill. Numerous synthetic modifications of the ginkgolide parent compounds have been carried out. Methods for obtaining synthetic derivatives of the ginkgolides have been described in, for example, U.S. Patent Nos. 6,693,091 and 5,541,183.

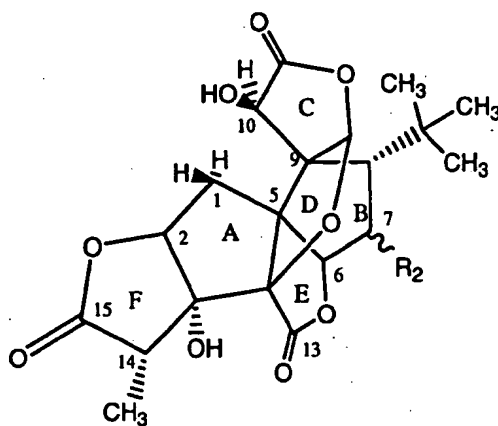
For example, two approaches have been developed for converting GC into GB, the most potent ginkgolide PAF receptor antagonist. First, Weinges and Schick described a four-step procedure in which the 1-OH group of GC was protected as a tert-butyldiphenylsilyl (TBDPS) ether prior to treatment with phenyl chlorothionoformate to give an intermediate. This compound was then treated with Bu<sub>3</sub>SnH and azobisisobutyronitrile (AIBN) in a Barton-McCombie alcohol deoxygenation, and GB was liberated by removal of the silyl protecting

group (Weinges *et al.* (1991) Liebigs Ann. Chem. 81). Similarly, Corey *et al.* protected GC as a benzyloxymethyl (BOM) ether at the hydroxyl at position 10, and followed a similar path to form GB (see Corey *et al.* (1992) Tetrahedron Lett. 33:6955). A very convenient, two-step procedure was described by Teng (Teng (1995) GB 2288599 and Chem. Abstr. 124: 117659) in which GC was treated with triflic anhydride to yield exclusively 7-O-triflate-GC, which was reduced with  $\text{Bu}_4\text{NBH}_4$  to give GB.

The different reactivities of the 1-, 7-, and 10-OH groups of the ginkgolides are noteworthy: A bulky silyl group reacts preferentially at 1-OH, whereas all alkylation reagents react at 10-OH and triflic anhydride reacts exclusively at 7-OH of GC under strongly acidic conditions (Jaracz *et al.* (2002) J. Org. Chem. 67:4623). Generally, 1- and 10-OH are the most reactive OH groups owing to the hydrogen bonding between the two and hence the relative ease of formation of a delocalized alkoxy anion (Corey *et al.* (1992) Tetrahedron Lett. 33:6955; Jaracz *et al.* (2002) J. Org. Chem. 67:4623). The increased reactivity of 7-OH towards sulfur nucleophiles may be due to the soft-atom nature of sulfur. Accordingly, sulfur nucleophiles are particularly useful in the synthesis of certain Ginkgolide J derivatives.

During the structural studies of ginkgolides and bilobalide, several analogues were synthesized. For example, acetylated ginkgolides and bilobalide (Nakanishi *et al.* (1971) J. Am. Chem. Soc. 93:3544; and Weinges *et al.* (1972) Justus Liebigs Ann. Chem. 759, 158) were described, as well as the iso-derivatives, which stem from a translactonization of ring E in ginkgolides. Recently, an X-ray crystal structure of the iso-derivative 1,6,10-triacetate-isoGC was obtained, and a mechanism for the formation of iso-derivatives that includes opening of ring E, stabilization of the intermediate by hydrogen bonding to 3-OH, and capture by acetic anhydride was suggested (Jaracz *et al.* (2002) J. Org. Chem. 67:4623). A similar translactonization was described by Weinges *et al.* upon acetylation of BB and during the preparation of various acyl derivatives of BB (Weinges *et al.* (1987) Liebigs Ann. Chem. 1079). Weinges *et al.* has carried out numerous synthetic studies of ginkgolides and bilobalide (Weinges *et al.* (1986) Liebigs Ann. Chem. 1057) (Weinges *et al.* (1993) Liebigs Ann. Chem. 1023) (Weinges *et al.* (1993) Liebigs Ann. Chem. 287) (Weinges *et al.* (1997) Ann. Chem. 1607) (Weinges *et al.* (1997) Ann. Chem. 1755) (Weinges *et al.* (1997) Ann. Chem. 991) (see also, the preparation of radiolabeled analogues of ginkgolides, in this case [ $^{14}\text{C}$ ] GA (Weinges *et al.* (2000) Tetrahedron 56:3173)).

When ginkgolides are tested in pharmacological assays it may be necessary to make a concentrated stock solution of the compound in dimethyl sulfoxide (DMSO), owing to their low solubility in water. DMSO may cause problems in certain assay systems, (MacIennan *et al.* (1996) Neurosci. Res. 26:395) although such effects are not uniformly observed (Strømgaard *et al.* (2002) J. Med. Chem. 45:4038). To increase the water solubility of ginkgolides, Weber and Vasella synthesized glycosylated ginkgolides analogues (Weber *et al.* (1997) Helv. Chim. Acta 80:2352) (Vasella *et al.* (1998) (SCRAS) US 6143725 Chem. Abstr. 130:38639). Glycosidation was carried out by reaction of ginkgolides with a glycosylated analogues. Intra- and intermolecular bonds of these analogues, as well as the parent ginkgolides, were studied by  $^1\text{H}$  NMR spectroscopic analysis (Bernet *et al.* (2000) Helv. Chim. Acta 83:995).



[8]

Ginkgolides contain three lactones (designated rings C, E, and F) (see structure [8] above, which shows the ring nomenclature scheme for the generalized GA/GJ ginkgolide structure as well as the carbon atom numbering scheme used in ginkgolide nomenclature) and hence their structures in solution are highly dependent on the pH value of the media in which they are dissolved. Zekri *et al.* determined the ionization constants of ginkgolides by  $^1\text{H}$  NMR titrations (Zekri *et al.* (1996) Anal. Chem. 68:2598), which showed that the lactones start to open at around pH 7. At pH 8, the predominant species ( $\approx 50\%$ ) is the form in which only ring F is open, ring E is open to a lesser extent ( $\approx 20\%$ ). An increase in the pH value to 10 opens both rings E and F ( $\approx 90\%$ ). Only at pH 13 is the species with all the lactones open present ( $\approx 40\%$ ), although about 60% is still in the form with only rings E and F open. Chemical schemes for the opening and/or removal of one or more of these ginkgolide lactone rings have been described

(Hu *et al.* (2001) J. Asian Nat. Prod. Res. 3: 219-22; Corey *et al.* (1989) Tetrahedron Lett. 30: 6959-62; and Weinges *et al.* (1997) Liebigs Ann. Chem. 1997: 991-3).

For example, Hu *et al.* ((2001) J. Asian Nat. Prod. Res. 3: 219-22) demonstrate the synthesis and biological activity of amide analogues of GA. Briefly, GA is treated with Jones reagent (an acidic aqueous solution containing chromic acid), which is added to an acetone solution of the GA to be oxidized. This treatment yields 10-oxoginkgolide, which is in equilibrium with its ring-C-open analogue, and a novel heptacyclic ginkgolide analogue, the formation of which is formed as a result of the formation of radicals from the t-butyl methyls of 10-oxoginkgolide A under sunlight irradiation. Reaction of 10-oxoginkgolide A with benzylamine in the THF solution and refluxing for 2 hours resulted in the formation of an amide derivative of the C-ring opened form of GA in which the PhCH<sub>2</sub>NH- group was connected to C<sub>11</sub> to form benzyl 11-ginkgamide. A similar strategy was employed to synthesize other amide derivative of ginkgolide A from 10-oxoginkgolide A and an appropriate amine using the same method (*i.e.*, in addition to the C<sub>11</sub>-NH-CH<sub>2</sub>Ph amide, a C<sub>11</sub>-NH-Ph amide (phenyl 11-ginkgamide), a C<sub>11</sub>-NH-CH<sub>3</sub> amide (methyl 11-ginkgamide), a C<sub>11</sub>-NH<sub>2</sub> amide (11-ginkgamide), a C<sub>11</sub>-NH-OH (hydroxyl 11-ginkgamide), and a morpholino-11-ginkgamide ginkgolide derivative were synthesized. Notably, these ring-C-open forms of GA (including amide and monoacid forms) retained substantial amounts of biological activity in a PAF-induced (platelet activating factor-induced) platelet aggregation assay IC<sub>50</sub> (uM) (Hu *et al.* (2001) J. Asian Nat. Prod. Res. 3: 219-22). Analogous strategies may be employed to obtain the C-ring opened, monoacid and analogous amide forms of GJ.

"Simple Analogs" of ginkgolide B that are missing the "F" ring structure have been synthesized, and shown to retain high levels of biological activity as antagonists of platelet activating factor (Corey and Gavai (1989) Tetrahedron Lett. 30: 6959-62). Briefly, the synthesis of these "simple analogs" of ginkgolide molecules with anti-PAF activity was the tetracyclic lactone, a key intermediate in the total synthesis of ginkgolide B described previously. (Corey *et al.* (1988) J. Am. Chem. 110:649; and Corey *et al.* (1988) Tetrahedron Letters 29:3201). The racemic form of the tetracyclic lactone was employed since a sizeable quantity of this compound was available from earlier work and all of the analogs of the tetracyclic lactone described here were obtained as racemates. Initial studies of the anti-PAF activity of early-stage, tetracyclic synthetic intermediates lacking the oxygen bridge between C(4) and C(12) had indicated very

low biological potency ( $IC_{50} > 100 \mu M$ ). In contrast, the lactone subunit attached to C(2) and C(3) of GB is not essential to biological activity, as indicated by the information which follows.

The tetracyclic lactone was transformed in five steps via to the chlorohydrin bis-lactone, by the following chemical synthetic sequence: (1) stereospecific  $\alpha$ -epoxidation of the C(1) – C(2) olefinic linkage (*m*-chloroperoxybenzoic acid in  $CH_2Cl_2$ , pH 8 aqueous phosphate buffer at  $23^\circ C$ , 92%); (2) oxirane ring opening to form a chlorohydrin intermediate (3 equiv of  $BCl_3$  and 4 equiv. of benzyltriethylammonium chloride in  $CH_2Cl_2$  at  $-45^\circ C$  to  $23^\circ C$ , 79%); (3) elimination of methanol to convert the methyl acetal intermediate to the corresponding dihydrofuran (heating with 5 equiv. of each pyridinium tosylate and pyridine in chlorobenzene at  $135^\circ C$  for 16 h, 83%); (4) dihydroxylation of the C(10) – C(11) olefinic linkage (osmium tetroxide-pyridine,  $55^\circ C$  for 36 h, 69%); and (5) oxidation of lactol to lactone ( $I_2$ , aqueous MeOH containing  $CaCO_3$  at  $23^\circ$  for 0.5 h, 82%). The resulting bis lactone diol was also converted to the corresponding bis methoxymethyl (MOM) ether, (excess  $CH_2(OMe)_2$ ,  $P_2O_5$  in  $ClCH_2CH_2Cl$  at  $23^\circ C$ , 69%). The anti-PAF activity of this intermediate was measured to be  $IC_{50} = 0.3 \mu M$  as compared to  $IC_{50} = 0.6 \mu M$  for ginkgolide B as a control. The diol intermediate ( $IC_{50} = 1.1 \mu M$ ) was somewhat less active than the bis MOM derivative, indicating that free hydroxyl groups are not necessary for anti-PAF biological activity of ginkgolides. Assuming that only one enantiomer of the MOM derivative is active, it follows that chiral MOM derivative is about four times more potent as an anti-PAF agent than ginkgolide B.

The 2-bromo analogs of the diol intermediate and the bis MOM derivative were synthesized from the tetracyclic lactone in a parallel fashion, and their anti-PAF  $IC_{50}$  values were determined as  $14 \mu M$  and  $0.6 \mu M$ , respectively.

Two  $1\alpha$ ,  $2\beta$ -dichloro derivatives (with either C(10)-OH or C(10)- $OCH_2OCH_3$ ), were also synthesized individually from the tetracyclic lactone by a sequence consisting of  $1\alpha$ ,  $2\beta$ -dichlorination (chlorine and benzyltriethylammonium chloride in  $CH_2Cl_2$ - $CF_3CH_2OH$  at  $0^\circ C$ , 65%) and then functional group modification at C(10) and C(11) as described above for the synthesis of the diol intermediate and the bis MOM derivative. The anti-PAF  $IC_{50}$  values of the two  $1\alpha$ ,  $2\beta$ -dichloro derivatives (having C(10)-OH and  $-OCH_2OCH_3$ ), were determined to be  $0.4 \mu M$  and  $0.2 \mu M$ , respectively. Thus, the active dichloro derivative enantiomer having C(10)- $OCH_2OCH_3$  is expected to be *ca.* six times as active as ginkgolide B with  $IC_{50} = 0.1 \mu M$ . Since

this dichloro enantiomer is considerably less polar than ginkgolide B, it is expected to be much better absorbed after p.o. administration, and possibly more efficacious.

The C(10) epimer of 1 $\alpha$ , 2 $\beta$ -dichloro derivatives (with C(10)-OH) was synthesized by oxidation to the corresponding  $\alpha$ -keto lactone (Jones' reagent, acetone-water, 23°C for 1 h) and subsequent reduction using excess aluminum amalgam in 20:1 THF-H<sub>2</sub>O at 23°C for 2 h. The anti-PAF IC<sub>50</sub> value for the C(10)-epimer of this dichloro enantiomer was found to be 1.3  $\mu$ M. The isomer of this dichloride having an oxygen bridge between C(4) and C(8), was synthesized from the related C(1) – C(2) – olefin (Corey *et al.* (1988) J. Am. Chem. 110:649) and found to be considerably less active, IC<sub>50</sub> = 38  $\mu$ M.

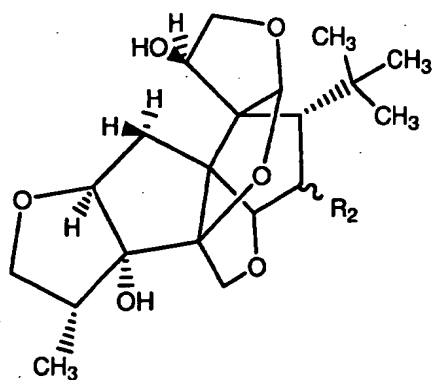
A carbonyl function at C(11) is beneficial for anti-PAF activity, but not essential; thus, the IC<sub>50</sub> for the tetracyclic lactone was 120  $\mu$ M as compared to 80  $\mu$ M for the corresponding structure having a carbonyl group at C(11).

The effect of substituents at C(10) was evaluated for the series several related derivatives, having no substituents at C(1) and C(2). The following IC<sub>50</sub> values were exemplary for those derivatives: 76  $\mu$ M; 13  $\mu$ M; 9.4  $\mu$ M; 13  $\mu$ M; 11  $\mu$ M; and 21  $\mu$ M. The  $\alpha$ -keto lactone obtained by oxidation of the 10-hydroxyl of some of these related derivatives, which showed an IC<sub>50</sub> of 18  $\mu$ M, upon irradiation, produced a related photoproduct structure IC<sub>50</sub> = 9.2  $\mu$ M. (Nakadaira *et al.* (1969) J. Chem. Soc. Chem. Commun. 1469) For comparison the IC<sub>50</sub> values for ginkgolides A, its 10-keto analog, and the photoproduct of the 10-keto analog (Nakadaira *et al.* (1969) J. Chem. Soc. Chem. Commun. 1469) were found to be 1.9  $\mu$ M, 3.9  $\mu$ M and 0.7  $\mu$ M, respectively. Similar strategies may be employed to obtain the analogous F-ring eliminated, "simple analogs" of GA and GJ.

In addition Weinges *et al.* ((1997) Liebigs Ann. Recueil. 1997: 991-3) have described an "F" ring opened form monoacid derivative of GA. Briefly, hydrogenation (in glacial acetic acid with Pt as catalyst) of 3,14-didehydro-10-hydroxyginkgolide (3, 14-anhydroginkgolide A), resulted in the formation of 3,14-didehydro-10-hydroxyginkgolide-15-acid. Further reaction with diazomethane led to the methyl ester, which was transformed into its dimethoxymethane (MOM) derivative form. Ozonolysis of the dimethoxymethane (MOM) derivative form resulted in the formation of a stable secondary ozonide, as well as 10-methoxymethoxy-3-oxo-14,15,16-trinorginkgolide.

Analogous strategies may be employed to obtain the F-ring opened, monoacid and structural analogue forms of GJ.

In addition to the opening and removing of lactone rings of the ginkgolides, the synthesis of ginkgolide triether, by reduction of the three carbonyls of the lactone rings, has been described (see Nakanishi *et al.* (1974) Natural Products Chemistry 1: 295-300; and Maruyama *et al.* (1967) Tetrahedron Lett. 1967: 303-8). The generalized structure of the ginkgolide triether is shown below as structure [9].



Briefly, Nakanishi *et al.* describe the reduction of GA at all three carbonyls to the corresponding circular triether by reduction when the syrup (an octaol), resulting from LAH (Lithium Aluminum Hydride) reduction of GA, is further subjected to pyrolysis by heating. In addition, Maruyama *et al.* have described the synthesis of GA triether ((1967) Tetrahedron Lett. 1967: 303-8) by lithium aluminum hydride reduction of GA followed by pyrolysis of the product to yield the "GA triether" (in fact, actually a tetraether counting the original ether ring of GA).

In addition to ring opened (and deleted), and triether forms of the ginkgolides, a number of reports of the synthesis of ginkgolide derivatives having modifications at position C-7 having the chirality of that designated for both GA derivatives and GJ derivatives, have been described (see Teng, (1991) U.S. Patent No. 5,241,084; Cazaux *et al.* (1995) (GB 2288599); Jaracz *et al.* (2004) Chem.- A Europ. J. 10: 1303 and Jaracz *et al.* (2002) J. Org. Chem. 67:4623-26). These methods may be applied to obtain certain of the GA and GC derivatives of the invention having a substituted "R<sub>2</sub>" group (with either a GA derivative or a GJ derivative chirality) at position C-7).

For example, U.S. Patent No. 5,241,084 describes a process for preparing GB from GC. This method may be applied to the conversion of GJ to GA. Furthermore, GB 2288599 describes a two-step process for converting GC to GB that involves first reacting ginkgolide C with a sulphonic anhydride to obtain 7-sulphonyloxy-ginkgolide B, and, in the second step, reacting the 7-sulphonyloxy-ginkgolide B with borohydride to eliminate the 7-sulphonyloxy group and thus produce ginkgolide B.

In addition, Vogensen *et al.* ((2003) J. Med. Chem. 46: 601-8) describe methods for synthesizing a series of ginkgolide derivatives having modifications at the C-7 position. The chirality of the C-7 atom here is described in terms of  $\alpha$  and  $\beta$  forms, whereby the  $\beta$  form corresponds to the Ginkgolide J derivative chiral form at C-7 described herein, with  $R_2$  substitutions having the R chiral form, while the  $\alpha$  form corresponds to the Ginkgolide A derivative chiral form, as described herein, with  $R_2$  substituents having the S chiral form. Briefly, various  $R_2$  substitutions at C(7) and in the alpha configuration, corresponding to GA derivatives of the invention, were synthesized by reacting GC with trifluoromethanesulfonic (Tf) anhydride which reacted with remarkable selectivity at the C(7)-OH (higher selective reactivity of C(7)-OH was also observed under strong acidic conditions). The resulting 7 $\beta$ -OTf-GB intermediate was then reacted with various nucleophiles to give the corresponding C-7 substituted derivatives. For example, reaction with sodium acetate yielded 7 $\alpha$ -O-acetate-GB; reaction with sodium phenyl acetate yielded 7 $\alpha$ -O-phenylacetate-GB; reaction with sodium azide yielded 7 $\alpha$ -azido-GB; reaction with tetrabutylammonium fluoride hydrate yielded 7 $\alpha$ -fluoro-GB; reaction with tetrabutylammonium chloride yielded 7 $\alpha$ -chloro-GB. In addition, a similar chemical approach was used to produce 7 $\alpha$ -N-methylamino-GB, 7 $\alpha$ -ethylamino-GB, and 7 $\alpha$ -ethylamino-GB.

Additional methods for synthesizing GA and GJ derivatives are described in Jaracz *et al.* ((2004) Chem. - A Europ. J. 10: 1303), which discloses methods to prepare various ether-, ester-, and carbonyl- derivatives of ginkgolides by selective derivitization of the hydroxyl groups, including the C(7)-OH of GJ. Accordingly, these methods may be applied to the synthesis of GJ derivatives having the R chiral form, as defined herein (or the  $\beta$ -configuration at C-7). In brief, the method includes selective alkylation at C(10)-OH with various benzyl-derived halides under mild reaction conditions to yield corresponding C(10)-OR blocked intermediates. Next, the C-7 position is derivatized as desired by esterification in the presence of pyridine.



Still other methods for synthesizing GA and GJ derivatives are described in, Jaracz *et al.* ((2002) J. Org. Chem. 67:4623-26), which discloses methods for making selective acetylations and other modifications to ginkgolides, including acetylations at C(7)-OH. Their results demonstrated the differences in reactivity among the hydroxyl groups of GC. Acetylation in the presence of base led to higher reactivity of 10-OH, while the pattern for acid-catalyzed acetylation of GC is different as seen in the case of H<sub>2</sub>SO<sub>4</sub>-catalyzed acetylation reaction. These observations can be explained by the hard-soft and acid-base (HSAB) principle: due to the hydrogen bonding between 1-OH and 10-OH, it is easy to form a delocalized alkoxy anion at 1-OH and 10-OH in the presence of a relatively mild base. This anion becomes a hard base compared to 7-OH. In the presence of acid, the alkoxy anion at 1-OH and 10-OH cannot be formed and 7-OH becomes the harder base, and thus more reactive toward acetylation. Further rational chemical synthetic strategies for synthesizing C(7)-altered ginkgolide derivatives are thereby supported. In addition, Hu *et al.* ((2000) Bioorg. Medic. Chem. 8: 1515-21) describe methods for synthesizing alkyl and alkoxycarbonyl derivatives of ginkgolide (including those having C(7)-OH that are prepared in one step through alkylation and acylation).

In addition to these methods for chemical synthesis of ginkgolide derivatives, U.S. Patent No. 6,693,091 describes analogs of terpe trilactones, including ginkgolide derivatives and methods for their synthesis (see also Stromgaard *et al.* (2002) J Med. Chem. 45: 4038-46). U.S. Patent No. 5,541,183, describes ginkgolide derivatives, including those having C(10)-OR substitutions and/or C(1)-OR substitutions. Furthermore, U.S. Patent No. 5,466,829 describes certain ginkgolide derivatives and processes for preparing them. U.S. Patent No. 6,143,725 describes the synthesis of a number of glycosylated ginkgolide derivatives. U.S. Patent No. 5,599,950 describes methods for converting GC into GA, which chemical methodologies can be applied to the synthesis of GA and GJ derivatives as well (see also U.S. Patent No. 5,241,084, described above). As with all the above cited references, the entire content of each of these cited U.S. Patents are hereby incorporated herein in their entirety.

#### 4.4 Antioxidants

The invention is directed to ginkgolide compositions, and methods of using such compositions, that may optionally include antioxidants in their formulation. Antioxidant agents which may be employed in accordance with the above-described methods include vitamin C,

vitamin E, N-acetyl-L-cysteine, resveratrol, coenzyme Q, alpha-lipoic acid, lycopene, or any combination thereof. In addition, antioxidant minerals, such as selenium, copper, zinc, manganese, and carotenoids, such as lutein, lycopene, or alpha- and/or beta-carotene may be utilized in the ginkgolide compositions and therapeutics of the invention. In particular, antioxidant agents such as vitamin A, vitamin C, and vitamin E (alpha-tocopherol and other active tocopherols), folic acid, ubiquinone (coenzyme Q10), 2, 3-dihydroxy benzoic acid, and 2,5-dihydroxy benzoic acid are useful antioxidants of the invention. Exemplary antioxidants, particularly for oral delivery of the ginkgolide compositions of the invention, are described in U.S. Patent Nos. 6, 551,629 and 6,572,899.

In another aspect, the ginkgolide compositions of the invention are optimized to prevent oxidation during use by including one or more antioxidant compounds. For example, vitamin C (supplied as in the particularly stable form of L-ascorbic acid-2-phosphate or in any other form) may be used. Examples of other antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like. Furthermore N-acetyl-L-cysteine, a potent antioxidant, which is also a precursor of L-cysteine and which thereby promotes the intracellular synthesis of glutathione, may be a particularly useful antioxidant to include in certain cell culture applications (*e.g.*, in vitro applications) of the invention. Other particularly useful antioxidants which may be included in cell culture applications are vitamin E (*e.g.*, D-alpha-tocopherol), resveratrol (a plant phytoalexin derived from, *e.g.*, grapes), coenzyme Q, alpha-lipoic acid, lycopene, bioflavonoids, and quercetin. The form and concentration of each such antioxidant is known in the art or may be discerned with routine testing. For example, L-ascorbic acid-2-phosphate may be used at a concentration of about 20  $\mu$ M to about 2 mM (*e.g.*, at 0.2 mM), and N-acetyl-L-cysteine may be used at a concentration of about 0.2 to about 20 mM (*e.g.*, at 2 mM).

In certain instances, the antioxidant may be a flavonoid, such as a flavonoid derived from a *Ginkgo biloba* or other plant extract, as is known in the art. Flavonoids, abundant polyphenols found in plants, are antioxidant molecules having a "flavan nucleus," and can be classified into ten groups based on differences in their chemical structures (see [www.herbalchem.net](http://www.herbalchem.net)). These

groups include anthocyanidins (*e.g.*, as found in grapes, bilberry blueberries and cocoa), and anthocyanins (*e.g.*, as found in elderberry), proanthocyanidins (*e.g.*, as found in red wine, blueberries, blackberries, and other red/blue/purple colored plant parts), flavanols (*e.g.*, flavan-3-ols) (found in green tea, cocoa powder, red wine, and motherwort), flavonols (*e.g.*, flavonol glycosides found in *Ginkgo biloba* including quercetin, kaempferol, and isorhamnetin), flavones (*e.g.*, as found in celery and red peppers), and flavanones (*e.g.*, as found in citrus plants), isoflavones (*e.g.*, genistein, as found in red clover, alfalfa, peas, soy and other legumes).

#### 4.5 Pharmaceutical Formulations

The ginkgolide compositions of the invention may be administered together with any suitable pharmaceutically acceptable carrier. Furthermore, the ginkgolide compositions of the invention may be formulated for administration in any convenient way (*e.g.*, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents which facilitate any means of administration). The ginkgolide compounds may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin; or (4) intravaginally or intrarectally, for example, as a pessary, cream or foam. However, in certain embodiments the subject compounds may be simply dissolved or suspended in sterile water.

The amount of a composition of the invention, which is effective in the treatment or prevention of neurological or neurodegenerative condition can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges (see Examples). The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and is decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

As described above, formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient, which can be combined with a carrier material to produce a single dosage form, will generally be that amount of the compound, which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about one per cent to about ninety-nine percent of active ingredient, particularly from about 5 per cent to about 70 per cent, most particularly from about 10 per cent to about 30 per cent.

The ginkgolide compositions of the invention may be administered together with any suitable pharmaceutically acceptable carrier, such as a pharmaceutically acceptable salt or excipient, *e.g.*, saline or distilled water. In certain instances, diffusion of the compositions of the invention may be facilitated by excipients such as salts, sugars or alcohols. The compound may be optionally administered as a pharmaceutically acceptable salt.

The formulations described herein may optionally include preservatives, such as antioxidants, that stabilize the ginkgolide, and thereby maintaining therapeutic activity. Examples of pharmaceutically-acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Furthermore, excipients such as salts, sugars and alcohols, may optionally be used to facilitate diffusion of the ginkgolide therapeutic. Non-limiting representative excipients that can be used in combination with the present invention include saccharides, such as sucrose, trehalose, lactose, fructose, galactose, mannitol, dextran and glucose; poly alcohols, such as glycerol or sorbitol; proteins, such as albumin; hydrophobic molecules, such as oils; and hydrophilic polymers, such as polyethylene glycol, among others. Pharmaceutical formulations of compounds of the invention described herein includes isomers such as diastereomers and

enantiomers, mixtures of isomers, including racemic mixtures, salts, solvates, and polymorphs thereof.

In certain cases, the compounds of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically-acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of compounds of the present invention. These salts can likewise be prepared in situ during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form with a suitable base, such as, but not limited to, the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, with ammonia, or with a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Non-limiting representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

The pharmaceutically-acceptable carrier may be a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject ginkgolide compositions from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include, but are not limited to: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate, magnesium stearate, and polyethylene oxide-polypropylene oxide copolymer as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Therapeutic formulations may be in the form of liquid solutions or suspensions. Methods well known in the art for making formulations are found, for example, in Remington: The Science and Practice of Pharmacy (20th ed., ed. A.R. Gennaro AR.), Lippincott Williams & Wilkins, 2000. For oral administration, formulations may be in the form of tablets or capsules. Intranasal formulations may be in the form of powders, nasal drops, or aerosols. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated naphthalenes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycolate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel. The concentration of the compound in the formulation will vary depending upon a number of factors, including the dosage of the drug to be administered, and the route of administration.

The ginkgolide composition may be encapsulated within or administered with a biocompatible polymer to provide controlled release of the ginkgolide compound(s). The biocompatible polymer can be either a biodegradable polymer or a biocompatible non-degradable polymer which releases over time the incorporated ginkgolide composition by diffusion. The ginkgolide composition can be homogeneously or heterogeneously distributed within the biocompatible polymer. A variety of biocompatible polymers are useful in the practice of the invention, the choice of the polymer depending on the rate of ginkgolide composition release required in a particular treatment regimen. The ginkgolide composition can be provided in a polymeric sustained release formulation in which the amount of ginkgolide composition in the composition varies from about 0.1% to about 30%, from about 0.1% to about 10%, or from about 0.5% to about 5% (w/w).

Non-limiting representative synthetic, biodegradable polymers include, for example: polyamides such as poly (amino acids) and poly (peptides); polyesters such as poly (lactic acid), poly (glycolic acid), poly (lactic-co-glycolic acid), and poly (caprolactone); poly (anhydrides); polyorthoesters; polycarbonates; and chemical derivatives thereof (substitutions, additions of

chemical groups (*e.g.*, alkyl, alkylene), hydroxylations, oxidations, and other modifications routinely made by those skilled in the art), copolymers and mixtures thereof. The degradable sustained released composition can have a half-life for the release of the ginkgolide composition of greater than one week, two weeks, three weeks, one month, two months, three months, or four months when placed in the body, or otherwise in contact with cells (*e.g.*, neurons) to be treated.

The ginkgolide composition can also be encapsulated within a biocompatible non-degradable polymer. Non-limiting representative non-degradable polymers include polysaccharides; polyethers, such as poly (ethylene oxide), poly (ethylene glycol), and poly (tetramethylene oxide); vinyl polymers, such as polyacrylates, acrylic acids, poly (vinyl alcohol), poly (vinyl pyrrolidone), and poly (vinyl acetate); polyurethanes; cellulose-based polymers, such as cellulose, alkyl cellulose, hydroxyalkyl cellulose, cellulose ethers, cellulose esters, nitrocellulose, and cellulose acetates; polysiloxanes and other silicone derivatives.

Useful polymeric sustained released compositions are a solid particulate having an average diameter of less than 400  $\mu\text{m}$ , 200  $\mu\text{m}$ , 100  $\mu\text{m}$ , or 50  $\mu\text{m}$ .

Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the ginkgolide composition is optionally mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose,

mannitol, and/or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, sodium carbonate, and sodium starch glycolate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol, glycerol monostearate, and polyethylene oxide-polypropylene oxide copolymer; absorbents, such as kaolin and bentonite clay; lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made, by molding, in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The invention also provides kits comprising one or more containers filled with one or more of the ginkgolide compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products.

#### 4.6 Therapeutic Applications

This invention provides therapeutic methods and compositions useful for treating neurodegeneration and loss of cognitive function. The methods of the invention involve administering to a subject a therapeutic compound which effects neuroprotection and/or cognition enhancement. Neuroprotection and/or cognition enhancement can be effected, for example, by blocking neurotoxicity associated with protein aggregate formation (e.g. amyloid



beta plaque formation), or by blocking the inhibition of neuronal long-term potentiation that is associated with such protein aggregate formation.

Many neurodegenerative diseases, including Alzheimer's and Parkinson's and the transmissible spongiform encephalopathies (prion diseases), are characterized by neuronal loss and the accumulation of protein aggregates that are typically fibrillar in structure. Huntington's disease, a hereditary neurodegenerative disease, is characterized by the accumulation of nuclear protein inclusion in the brain (Taylor *et al.* (2002) Science 296: 1991-5; Ross (2002) Neuron 35: 819-22) and huntingtin, the affected protein, has been shown to form amyloid fibrils in vitro (Scherzinger *et al.* (1997) Cell 90: 549-58). Indeed many neurodegenerative diseases have been found to be associated with the accumulation of apparently neurotoxic protein aggregates including: amyotrophic lateral sclerosis, which is characterized by the intraneuronal inclusions of insoluble superoxide dismutase protein; transmissible spongiform encephalopathies, which are characterized by accumulation of extracellular plaques of protease-resistant prion protein (PrP); fronto-temporal dementia, which is characterized by accumulation of neurofibrillary tangles of fibrillar tau protein; diffuse Lewy body disease, which is characterized by cortical Lewy bodies of fibrillar alpha-synuclein, familial British dementia, which is characterized by extracellular amyloid plaques of fibrillar Aβ protein; familial Danish dementia, which is characterized by accumulation of extracellular plaques of insoluble Aβ protein; and spinocerebellar ataxia, which is characterized by the appearance of intranuclear neuronal inclusions see Caughey and Lansbury (2003) Annu. Rev. Neurosci. 26: 267-98). Remarkably, the accumulation of neurotoxic protein deposits, such as amyloid beta and tau, even appears to occur in brain injury, such as occurs from traumatic head injury (Franz *et al.* (2004) Neurology 60: 1457-61; Lambri *et al.* (2001) Clin. Neuropathol. 20: 263-71). Notably, even Down's syndrome is characterized by the expression of abnormally high levels of the amyloid precursor protein (APP) and its product the amyloid-beta protein (Lemere *et al.* (1996) Neurobiol. Dis. 3: 16-32). Therefore protein aggregation appears to be a common widespread cause of neurodegeneration in a multitude of otherwise clinically distinct neurological disease and disorders. Indeed, the ability of such protein aggregation to directly cause neurodegenerative neuronal cell death is supported by a wealth of scientific evidence including: (i) pathology (the colocalization of neuronal cell death and protein aggregates); (ii) genetics (the genes linked to familial forms of these disease encode the aggregated protein); (iii) animal modeling (overexpression of the aggregated proteins

produces disease-associated phenotypes (Wong *et al.* (2002) Nat. Neurosci. 5: 633-39); (iv) biophysics (disease-associated mutations promote *in vitro* aggregation (Jarret and Lansbury (1993) Cell 75: 1055-58); and (v) mathematical modeling (rates of cell death (Clarke *et al.* (2000) Nature 406: 195-99) and disease onset and progression are consistent with a nucleation-dependent aggregation process (Perutz and Windle (2001) Nature 412: 143-44; Eigen (1996) Biophys. Chem. 63: A1-18). Accordingly, protein aggregate-induce cell death is a common essential link in neurodegenerative disease.

Significantly, protein aggregates play a role in, not only neurotoxicity, but also the reversible loss of cognitive functions associated with inhibition of neuronal long-term potentiation (LTP) (reviewed in Kourie (2001) Cell. Mol. Neurobiol. 21: 173-213; and Turner *et al.* (2003) Prog. Neurobiol. 70: 1-32). LTP is a form of synaptic information storage that is a well-established central process for memory mechanisms. The inhibition of neuronal LTP by aggregatable proteins such amyloid beta protein, has been associated with reversible memory loss (Gong *et al.* (2003) PNAS 100: 10417-22). Notably, neuronal LTP inhibition is distinct from neurotoxicity due to protein aggregation. In particular, neuronal LTP effects have a rapid onset without significantly affecting basal synaptic transmission, and there are amyloid beta peptide mutations which uncouple the two effects – *i.e.*, substitutions at  $\beta$ A position 35 block neurotoxicity, but not LTP inhibition (Chen *et al.* (2000) Neurosci Res. 60: 65-72).

The invention provides ginkgolide compositions that are capable of inhibiting, blocking or reversing both the neurotoxicity resulting from abnormal protein aggregates, and the inhibition of neuronal long-term potentiation associated with such aggregatable proteins. The invention thereby provides compositions and methods for treating or preventing otherwise irreversible neurodegenerative diseases and disorders, as well as for treating or preventing reversible cognition-impairment, such as memory impairment (including age-related memory impairment) and learning disorders.

The invention thereby provides ginkgolide compositions and associated methods for treating patients with a neurological or neurodegenerative disease or disorder such as Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, Alpers' disease, corticobasal ganglionic degeneration, multiple system atrophy, motor neuron disease, olivopontocerebellar atrophy, Parkinson's disease, prion disease, Rett syndrome, tuberous sclerosis, Shy-Drager syndrome, Huntington's disease, Pick's disease, Creutzfeld-Jakob disease,

depression, aging, head trauma, stroke, CNS hypoxia, cerebral senility, multi-infarct dementia and other neurological conditions including acute neuronal diseases, senile dementia, epileptic dementia, presenile dementia, post-traumatic dementia, vascular dementia and post stroke dementia, alcoholism, meningitis, diabetic peripheral neuropathy, neonatal hypoxia, stroke, global cerebral ischemia, or any combination thereof. Examples of other neurodegenerative conditions subject to therapeutic treatments of the invention include acute neurodegenerative disorders such as stroke, traumatic brain injury, schizophrenia, peripheral nerve damage, hypoglycemia, spinal cord injury, epilepsy, and anoxia and hypoxia. In general, the subject neurodegenerative disorders for treatment include any disorder in which progressive loss of neurons occurs either in the peripheral nervous system or in the central nervous system.

The invention also provides methods of treating neurological conditions and/or preventing an undesirable mental condition (*e.g.*, memory loss) by administering to a subject an effective amount of a therapeutic ginkgolide compound capable of mitigating neurodegeneration. In addition, the subject ginkgolides, and associated methods, provide beneficial neuroprotective effects. Neuroprotection is the inhibition of progressive deterioration of neurons that leads to nerve cell death. Therefore, in the methods of the invention, neurodegeneration in a subject is mitigated, and/or neuroprotection and/or cognition enhancement is effected, by administering a therapeutic compound of the invention to the subject.

In a particularly useful aspect, the invention provides ginkgolide compositions and methods for treating dementias. Dementias are diseases that include memory loss and additional intellectual impairment separate from memory. The invention provides methods for treating patients suffering from memory impairment in all forms of dementia. Dementias are classified according to their cause and include: neurodegenerative dementias (*e.g.*, Alzheimer's, Parkinson's disease, Huntington's disease, Pick's disease), vascular (*e.g.*, infarcts, hemorrhage, cardiac disorders), mixed vascular and Alzheimer's, bacterial meningitis, Creutzfeld-Jacob Disease, multiple sclerosis, traumatic (*e.g.*, subdural hematoma or traumatic brain injury), infectious (*e.g.*, HIV), genetic (down syndrome), toxic (*e.g.*, heavy metals, alcohol, some medications), metabolic (*e.g.*, vitamin B12 or folate deficiency), CNS hypoxia, Cushing's disease, psychiatric (*e.g.*, depression and schizophrenia), and hydrocephalus. The present invention includes methods for dealing with memory loss separate from dementia, including mild cognitive impairment (MCI) and age-related cognitive decline. The present invention

includes methods of treatment for memory impairment as a result of disease. In another application, the invention includes methods for dealing with memory loss resulting from the use of general anesthetics, chemotherapy, radiation treatment, post-surgical trauma, and therapeutic intervention.

The ginkgolide compositions of the invention are also useful in the treatment of depression. Depression can be the result of organic disease, secondary to stress associated with personal loss, or idiopathic in origin. There is a strong tendency for familial occurrence of some forms of depression suggesting a mechanistic cause for at least some forms of depression. The diagnosis of depression is made primarily by quantification of alterations in patients' mood. These evaluations of mood are generally performed by a physician or quantified by a neuropsychologist using validated rating scales, such as the Hamilton Depression Rating Scale or the Brief Psychiatric Rating Scale. Numerous other scales have been developed to quantify and measure the degree of mood alterations in patients with depression, such as insomnia, difficulty with concentration, lack of energy, feelings of worthlessness, and guilt. The standards for diagnosis of depression as well as all psychiatric diagnoses are collected in the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) referred to as the DSM-IV-R manual published by the American Psychiatric Association, 1994.

The ginkgolide compositions of the invention are further useful to improve or enhance memory in a normal subject, *e.g.*, a normal human subject without a neurological disease or disorder, or other form of neurological trauma. In certain applications, the ginkgolide compositions are used to enhance general cognitive function in such a normal human subject. In particular, the compositions of the invention promote neuronal long-term potentiation, a neurocellular function that is central to learning and memory. The ability of these compositions to improve memory may be further demonstrated in a variety of routine animal tests (see Staubli *et al.* (1994) Proc. Natl. Acad. Sci. 91: 777-781; and Arai *et al.* (1996) The Journal of Pharmacology and Experimental Therapeutics 278: 627-638).

Subjects for treatment with the ginkgolide compositions of the invention include living organisms in which the particular neurological condition to be treated can occur. Examples of subjects include humans, apes, monkeys, cows, sheep, goats, dogs, cats, mice, rats, and transgenic species thereof. As would be apparent to a person of skill in the art, the animal subjects employed in the working examples set forth below are reasonable models for human

subjects with respect to the tissues and biochemical pathways in question, and consequently the methods, therapeutic compounds and pharmaceutical compositions directed to same. Dosage forms for animals such as, for example, rats can be and are widely used directly to establish dosage levels in therapeutic applications in higher mammals, including humans.

The pharmacological agents of the invention may be advantageously shown to be neuroprotective in one or more animal models known in the art (see *e.g.*, van Leuve (2000) Prog. Neurobiol. 61: 305-12). Agents that are neuroprotective in such animal models may, accordingly, be applied in human therapeutics, after appropriate adjustment of dosage. Specifically, there are comparable memory-deficit patterns between brain-damaged rats and humans, which indicates that the rat can serve as an excellent animal model to evaluate the efficacy of pharmacological treatments or brain damage upon memory.

As would also be apparent to a person skilled in the art, the invention further encompasses methods of the invention employed *ex vivo* or *in vitro*. For example, the Examples describe studies utilizing excised neuronal tissue as well as isolated neurons. Furthermore, diagnostic tests or studies of efficacy of selected compounds may conveniently be performed *ex vivo* or *in vitro*, including in animal models. Such tests, studies and assays are within the scope of the invention.

Administration of the compositions of the present invention to a subject to be treated can be carried out using known procedures, at dosages and for periods of time effective to mitigate neurodegeneration, and/or to effect neuroprotection and/or cognition enhancement in the subject. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the amount of neurodegeneration that has already occurred at the clinical site in the subject, the age, sex, and weight of the subject, and the ability of the therapeutic compound to mitigate neurodegeneration and/or to effect neuroprotection and/or cognition enhancement in the subject. Dosage regimens can be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. For example, an effective dose range for a therapeutic compound of the invention may be between 0.5 and 500 mg/kg of body weight per day. In an aqueous composition, preferred concentrations for the active compound (*i.e.*, the therapeutic compound that can mitigate

neurodegeneration and/or effect neuroprotection and/or cognition enhancement) are between 5 and 500 mM, and between 10 and 100 mM.

The therapeutic compounds of the invention can be effective when administered orally. Accordingly, a preferred route of administration is oral administration. Alternatively, the active compound may be administered by other suitable routes such as transdermal, subcutaneous, intraocular, intravenous, intramuscular or intraperitoneal administration, and the like (*e.g.*, by injection). Depending on the route of administration, the active compound may be coated in a material to protect the compound from the action of acids, enzymes and other natural conditions which may inactivate the compound.

The compounds of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes certain highly hydrophilic compounds. In certain embodiments, the invention includes ginkgolide compositions containing hydrophilic flavonoid compounds. To ensure that certain of the therapeutic compounds of the invention cross the BBB, they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs ("targeting moieties"), thus providing targeted drug delivery. Exemplary targeting moieties include folate and biotin (see, *e.g.*, U.S. Pat. No. 5,416,016); antibodies; and surfactant protein A receptor. Accordingly, in certain instances, the therapeutic compounds of the invention are formulated in liposomes; and in particular instances, the liposomes include a targeting moiety.

Delivery and *in vivo* distribution can also be affected by alteration of a substituent group of a compound of the invention.

To administer the therapeutic compound by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the therapeutic compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan et al., 1984). The therapeutic compound may also be administered parenterally (*e.g.*, intramuscularly, intravenously, intraperitoneally, intraspinally, or intracerebrally). Dispersions can be prepared in glycerol,

liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms. Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The vehicle can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants.

Prevention of the action of microorganisms can be achieved by the addition of various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In some cases, it will be preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

Sterile injectable solutions can be prepared by incorporating the therapeutic compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the therapeutic compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yield a powder of the active ingredient (*i.e.*, the therapeutic compound) optionally plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The therapeutic compound can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The therapeutic compound and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly

into the subject's diet. For oral therapeutic administration, the therapeutic compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of the therapeutic compound in the compositions and preparations may, of course, be varied. The amount of the therapeutic compound in such therapeutically useful compositions is such that a suitable dosage will be obtained.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The a specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such a therapeutic compound for the treatment of neurological conditions in subjects.

Therapeutic compositions can be administered in time-release or depot form, to obtain sustained release of the therapeutic compounds over time. The therapeutic compounds of the invention can also be administered transdermally (*e.g.*, by providing the therapeutic compound, with a suitable carrier, in patch form).

Active compounds are administered at a therapeutically effective dosage sufficient to mitigate neurodegeneration and/or to effect neuroprotection and/or cognition enhancement in a subject. A "therapeutically effective dosage" preferably mitigates neurodegeneration by about 20%, more preferably by about 40%, even more preferably by about 60%, and still more preferably by about 80% relative to untreated subjects. The ability of a compound to mitigate neurodegeneration can be evaluated in model systems that may be predictive of efficacy in mitigating neurodegeneration in human diseases, such as animal model systems known in the art (including, *e.g.*, the method of transient middle cerebral artery occlusion in the rat) or by *in vitro* methods, (including, *e.g.*, the assays described herein).

It will be appreciated that the ability of a compound of the invention to mitigate neurodegeneration will, in certain embodiments, be evaluated by observation of one or more symptoms or signs associated with neurodegeneration *in vivo*. Thus, for example, the ability of a



compound to mitigate neurodegeneration may be associated with an observable improvement in a clinical manifestation of the underlying neurodegeneration-related disease state or condition, or a slowing or delay in progression of symptoms of the condition. Thus, monitoring of clinical manifestations of disease can be useful in evaluating the neurodegeneration-mitigating efficacy of a compound of the invention.

#### 4.7 Compositions and Methods for Imaging

The invention provides compositions, and methods of use, of Ginkgolide A and Ginkgolide J, and derivatives thereof, for imaging (*e.g.*, diagnostic biomedical imaging in a human patient). U.S. Patent No. 6,693,091 describes the synthesis and use of analogs of the terpene trilactones from *Ginkgo biloba* for bioorganic and imaging studies.

In certain applications, one or more atoms of the GA, GJ or a derivative of GA or GJ may be labeled, *e.g.* by incorporation of a suitable radiolabel during chemical synthesis or by bioincorporation in a *Ginkgo biloba* plant. In other applications, a labeled, detectable by one or more imaging methods, may be incorporated at the "R<sub>2</sub>" group of Ginkgolide A or J. Suitable R<sub>2</sub> labels include photoactivatable moieties, fluorescent moieties, and radioactive moieties.

The invention thereby provides a process for detecting the binding of Ginkgolide A or Ginkgolide J, or a derivative thereof, to a target, by contacting the compound with the target and detecting the binding of the compound to the target. The target may be, for example, an enzyme or a receptor, particularly one which plays a role in neuronal cell survival and/or neuronal long-term potentiation processes.

This invention further provides a process for detecting the localization of a receptor in a subject by administering any of the described compounds to the subject and detecting at any location in the subject's body to identify a point of accumulation of the compound so as to thereby localize the receptor in the subject. Localization of a receptor means a higher concentration of that receptor than at other points in the subject's body. For example, particular receptors, so localized, may be those active in neuronal long-term potentiation and cell survival, and, more particularly, in the reversal of neuronal long-term potentiation resulting from beta-amyloid protein.

For example, the photoactivatable moieties may react with a receptor, enzyme or other target upon irradiation and enable researchers to identify the targets of compounds, to determine

the affinity and selectivity of the drug-target interaction, and to identify the binding site on the target. Examples are presented from three fundamentally different approaches: (1) photoaffinity labeling of target macromolecules; (2) photoactivation and release of "caged ligands"; and (3) photoimmobilization of ligands onto surfaces. A number of photoactivatable moieties are described in the literature, for example, aryl azides, which, when photoactivated to yield aryl nitrenes, can label any binding site containing carbon-hydrogen bonds by insertion into the C-H bond (Galaray, et al., J. Biol. Chem., 249: 350 (1974); U.S. Pat. No. 4,689,310; and U.S. Pat. No. 4,716,122); and a number of others are described in U.S. Pat. No. 6,077,698, the contents of which are hereby incorporated by reference.

The photoactivatable groups can be used for treatment as well as screening studies and diagnostics. Photoactivatable groups can be used to irreversibly bind compounds to their targets. Thus, the subject invention also provides compounds useful in methods of treatment where a desired compound is irreversibly bound to its target.

The photoactivatable groups may be phenylazides, purine and pyrimidine azides, 8-azidoadenosine, 2-azidoadenosine, diazoacetates, diazoketones, nitrobenzenes, aryldiazonium salts, or 3H,3-aryldiazirines. The preferred photoactivatable moieties for use with ginkgolides are benzophenone, trifluoromethyldiazirine and tetrafluorophenyl.

The fluorescent moiety, for example, may be 5-(dimethylamino)naphthalene-sulfonyl chloride (dansyl chloride), a fluorescent amine such as 1-pyrenemethylamine, or any number of other groups readily available from Molecular Probes--<http://www.probes.com/>, the contents of which are hereby incorporated by reference. Other specific groups which are useful in this invention are 1-(Bromoacetyl)pyrene, 3-Bromoacetyl-7-diethylaminocoumarin, 3-Bromomethyl-6,7-dimethoxycoumarin, 8-Bromomethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indace ne, 3-Bromomethyl-6,7-dimethoxy-1-methyl-2(<sup>1</sup>H)-quinoxazolinone, 6-Bromoacetyl-2-dimethylaminonaphthalene, and 4-(9-Anthroyloxy)phenacyl bromide.

Radioactive moieties are widely known in the art and include radionuclides, radionuclides covalently attached to other groups, and metal chelates. The appropriate ginkgolide-based radioligands can be prepared using known radioactive moieties to suit the environment of use and detection method. Gamma-emitter and positron-emitter radionuclides are well-known in the art and include <sup>111</sup>In, <sup>198</sup>Au, <sup>113</sup>Ag, <sup>111</sup>Ag, <sup>123</sup>I, <sup>125</sup>I, <sup>130</sup>I, <sup>131</sup>I, <sup>133</sup>I, <sup>135</sup>I, <sup>47</sup>Sc, <sup>72</sup>As, <sup>72</sup>Se, <sup>90</sup>Y, <sup>88</sup>Y, <sup>97</sup>Ru, <sup>100</sup>Pd, <sup>109</sup>Pd, <sup>105</sup>Rh, <sup>128</sup>Ba, <sup>197</sup>Hg, <sup>203</sup>Pb, <sup>212</sup>Pb, <sup>67</sup>Ga, <sup>68</sup>Ga, <sup>64</sup>

Cu, <sup>67</sup>Cr, <sup>97</sup>Ru, <sup>75</sup>Br, <sup>76</sup>Br, <sup>77</sup>Br, <sup>99m</sup>Tc, <sup>11</sup>C, <sup>13</sup>N, <sup>15</sup>O, <sup>3</sup>H and <sup>18</sup>F. For positron emission tomography (PET) studies contemplated by this disclosure, ginkgolide derivatives labeled with the radionuclides [<sup>18</sup>F]- and [<sup>11</sup>C] possessing half lives of 110 min and 20 min, respectively, are preferred. While [<sup>3</sup>H] is preferred for other radioactivity based studies.

## 5. EXAMPLES

Certain aspects of the invention are supported and further described in particular detail in the following illustrative examples.

### *Preparation of $\beta$ -amyloid 1-42*

In these experiments the toxic activity of the oligomeric form of  $\beta$ -amyloid 1-42 is examined. Among the different aggregation species of the peptide,  $\beta$ -amyloid 1-42 oligomers inhibit synaptic activity and induce cell death at lower concentrations than the fibrillar forms (Dahlgren *et al.* (2002) *J Biol Chem.* 277:32046-53; Stine *et al.* (2003) *J. Biol. Chem.* 278:11612-22). Briefly, the lyophilized peptide (American Peptide, Sunnyvale, CA) is re-suspended in 1,1,1,3,3,3-Hexafluoro-2-Propanol (HFIP; Sigma-Aldrich, St. Louis, MO) to 1mM using a glass gas-tight Hamilton syringe and divided in aliquots. The solution is then allowed to evaporate in the fume hood. The resulting clear peptide film is further dried under vacuum (6.7 mTorr) in a SpeedVac <sup>TM</sup> (Savant Instruments; Holbrook, NY). The dessicated pellet can be stored at -20°C for several months. Immediately prior to use, the aliquots are resuspended to 5mM in anhydrous dimethylsulfoxide (DMSO) by pipette mixing followed by bath sonication for 10 minutes. In order to obtain A $\beta$ 1-42 oligomers, 5mM A $\beta$ 1-42 is diluted in DMSO to 100 $\mu$ M in ice-cold culture medium, immediately followed by vortexing for 30 seconds and incubation at 4°C for 24 hours.

### 5.1 Terpene Trilactones Reverse beta Amyloid-Induced Inhibition of Long Term Potentiation

To determine whether terpene trilactones can rescue the inhibition of long-term potentiation (LTP) that is induced by beta-amyloid (1-42) protein, a terpene trilactone 70% enriched preparation (P8A) was used at a concentration of 200 ug/L in a hippocampal LTP assay system as described below.

To prepare hippocampal slices for LTP measurements, mice were decapitated, and their hippocampi were removed. Transverse hippocampal slices of a thickness of 400  $\mu$ m were

maintained in an interface chamber at 29°C. They were perfused with saline solution (124.0 mM NaCl, 4.4 mM KCL, 1.0 mM Na<sub>2</sub>HPO<sub>4</sub>, 25.0 mM NaHCO<sub>3</sub>, 2.0 mM CaCl<sub>2</sub>, 2.0 mM MgSO<sub>4</sub>, 10 mM glucose) continuously bubbled with 95 % O<sub>2</sub> and 5 % CO<sub>2</sub>. Slices were permitted to recover for at least 90 minutes before recordings. Further details of hippocampal slice preparation, as well as LTP measurement, have been described (see Son *et al.* (1998) Learn. Mem. 5: 231-45).

To make electrophysiological recording, a concentric bipolar platinum-iridium stimulation electrode and a low-resistance glass recording microelectrode filled with saline solution (5 millionohms resistance) were used. The extracellular field excitatory postsynaptic potential (fEPSP) measurements were recorded from the CA1 region of the hippocampus by placing both the stimulating and the recording electrodes in CA1 *stratum radiatum*. BST was assayed by plotting the stimulus voltage (V) against slopes of fEPSP to generate input-output relations. For LTP experiments, baseline stimulation was delivered every minute at an intensity that evoked a response approximately 35% of the maximum evoked response. Baseline response was recorded for 15 min. prior to beginning the experiment to assure stability of the response. LTP was induced using theta-burst stimulation (4 pulses at 100 Hz, with the bursts repeated at 5 Hz and each tetanus including 3 ten-burst trains separated by 15 seconds). Drugs or vehicle in 0.1 % DMSO were added to the bath solution for 20 min. prior to the induction of LTP. In the LTP blocking experiments, beta-amyloid protein was used to block tetanus-induced LTP by treating acute hippocampal slices with 200nM oligomeric beta-amyloid protein ( $\beta$ A) (amyloid-beta peptide 1-42 (DAEFRHDSGY EVHHQKL VFF AEDVGSNKGA IIGLMVGGVV IA)) for 30 minutes, with or without the addition of terpene trilactone or individual ginkgolide or bilobalide preparations.

The effect of the enriched terpene trilactone preparation (P8A) on beta-amyloid inhibition of tetanus-induced LTP in these hippocampal neuron preparations are shown in Figure 1. The horizontal bar indicates the period during which A $\beta$  (200nM) and P8A (200 $\mu$ g/L) were added to the bath solution. The arrows indicate the time at which the theta-burst stimulation was applied in this and the following figure. Notably, in this experiment, both beta-amyloid protein (A $\beta$ ) and terpene trilactone preparation (P8A) did not affect baseline transmission. The results show that amyloid-beta protein ( $\beta$ A) inhibited tetanus-induced LTP (black circles), but co-application of the terpene trilactone preparation (P8A) reversed the inhibition of LTP by amyloid-beta protein

( $\beta$ A) (black squares), while application of P8A alone had no effect on the baseline transmission (black diamonds).

The results support the ability of one or more ginkgolide terpene trilactone compositions to block the inhibition of long-term potentiation that is associated with beta-amyloid protein and that occurs in neurodegenerative and cognitive-impairing diseases and disorders (see section 4.6).

### 5.2 GA and GJ Alone Reverse beta Amyloid-Induced Inhibition of Long Term Potentiation

To determine which, if any, of the characterized components of the terpene trilactone preparation (P8A) mediate this neuroprotective effect, the effect of each of the four ginkgolides (GA, GB, GC, and GJ), as well as bilobalide (BB), was tested individually at a concentration of 1  $\mu$ M. The results show that Ginkgolide A and Ginkgolide J consistently rescued the LTP to the same values recorded in control untreated slices (Figure 2A), while Ginkgolides B and C and bilobalide did not (Figure 2B). In detail, Figure 2A is a summary graph showing that a 20 min. treatment with GJ and GA rescues L-LTP impairment in slices treated with A $\beta$  for 20 min. prior to L-LTP induction. The horizontal bar indicates the period during which these drugs were added to the bath solution. Figure 2B is a summary graph showing that 20 min. treatment with the ginkgolides GB and GC as well as the bilobalide BB does not rescue L-LTP impairment in slices treated with A $\beta$  for 20 min. prior to L-LTP induction. The horizontal bar indicates the period during which these drugs were added to the bath solution. Experiments in A and B were interleaved with each other. Therefore the components of the P8A terpene trilactone active in this neuroprotective effect were Ginkgolides A and J alone.

The results demonstrate the ability of Ginkgolide A and Ginkgolide J compositions to block the inhibition of long-term potentiation that is associated with beta-amyloid protein and that occurs in neurodegenerative and cognitive-impairing diseases and disorders (see section 4.6).

### 5.3 GJ Alone Protects Against beta Amyloid-Induced Neuronal Cell Death

To determine whether any of the ginkgolides or bilobalide are able to protect neurons against neurodegenerative cell death, rat hippocampal cultures kept 5-6 days *in vitro* where exposed for 24 hours to 5-10  $\mu$ M oligomeric beta amyloid 1-42 with or without the addition of

the terpende trilactone preparation (P8A), as well as with each of the ginkgolides or bilobalide at 1  $\mu$ M.

The results are shown in Figure 3. Hippocampal neuron cultures were prepared according to the method previously described (Vitolo *et al.* (2002) PNAS 99: 13217-21). Briefly, fetuses at embryonic day 18 (E18) from timed pregnant Sprague-Dawley rats (Taconic Farms) were sacrificed and the hippocampi removed. Neurons were then dissociated, plated on 24 well-plates coated with poly-L-lysine and maintained in a defined serum free medium. The obtained cultures resulted in a population enriched in large pyramidal neurons that constitute one of the primary targets of Alzheimer's disease. After 5-6 days in vitro cells were used for the experiments.

Cultures were treated adding 10  $\mu$ M beta amyloid 1-42 in its oligomeric form with or without the 70% enriched fraction of terpene trilactones (TTLs) at 50  $\mu$ g/ml, or alternatively each one of the ginkgolides (GA, GJ) at a concentration of 1  $\mu$ M. After 24h the number of viable cells was assessed using cell nuclei counting. Amyloid beta induced death of 50% of the cell population. Both the TTLs (P8a) and GJ, but not GA or any of the other ginkgolides (data not shown) were consistently able to prevent cell death (Student-Newman-Keuls multiple comparison test,  $p < 0.01$  for Ab vs P8a+Ab, \*,  $p < 0.05$  for Ab vs GJ+Ab, \*\*). In a preliminary experiment, a higher concentration of GJ (5  $\mu$ M) appeared to completely prevent amyloid beta toxicity. All the substances did not affect neuronal viability when added alone (data not shown). CTRL=Control, 100%. Ab = Amyloid beta, 48.5%  $\pm$  2.4. P8a+Ab = TTLs+Amyloid beta, 76.0  $\pm$  7.9. GA+Ab = Ginkgolide A+Amyloid beta, 50.5  $\pm$  5.7. GJ+Ab = Ginkgolide J+Amyloid beta, 70.7  $\pm$  1.4. Values represent mean  $\pm$  SEM of 3 consecutives experiments and each experiment was performed in triplicate. Ginkgolide J consistently protected the hippocampal cultures, which showed approximately 40% more cells than the other treatments.

The results support the ability of Ginkgolide J to prevent aggregatable protein-induced (*e.g.*,  $\beta$ A-induced) neuronal cell death that is associated with a multitude of neurodegenerative diseases and disorders (see section 4.6).

#### 5.4 Rapid Vesicle Cycling Assay

In this example, the cationic styrylpyridinium dye FM 1-43, is used to identify actively firing neurons and provide a facile means to assess the effect of new ginkgolide compositions of

the invention on neuronal function. The cationic styrylpyridinium dye FM 1-43 is a non-toxic, water-soluble dye that is non-fluorescent when in aqueous solution. Upon binding to the external leaflet of the cell membrane, however, it emits an intense fluorescent light. FM 1-43 is added to the medium of neuronal cultures, and it is rapidly internalized within recycling synaptic vesicles and stains pre-synaptic nerve terminals. Briefly, the cultured neurons are then stimulated by high  $K^+$  concentration, resulting in the release of neurotransmitter/s and the dye contained in the synaptic vesicle. As a consequence, the intensity of fluorescence in the nerve terminals decreases. The rate of exocytosis is measured as a decrease in fluorescence upon continued synaptic stimulation in dye-free medium.

The loading of FM 1-43 is induced by changing the incubation medium from physiological saline solution (119 mM NaCl, 2.5 mM KCl, 2 mM  $CaCl_2$ , 2 mM  $MgCl_2$ , 25 mM HEPES and 30 mM glucose) to hyperkalemic incubation solution (31.5 mM NaCl, 90 mM KCl, 2 mM  $CaCl_2$ , 2 mM  $MgCl_2$ , 25 mM HEPES and 30 mM glucose) with 5  $\mu$ M FM 1-43 for 45 seconds. The incubation solution is then changed back to physiological solution for 10 minutes to wash off the dye from the medium. In order to enhance the removal of FM 1-43 from the medium, the anionic cyclodextrin complexing agent ADVASEP-7 (1 mM, CyDex, Inc., Overland Park, KS) is added after 1 and 6 minutes of washing for 60 seconds. After a 10 minutes wash, which is enough to allow complete recycling and re-priming of the dye-stained population of synaptic vesicles, a fluorescent microscopic photo is taken to record the amount of fluorescence present in the nerve terminals. The cultures are then repeatedly exposed for 15 seconds to the hyperkalemic solution without the FM 1-43 to elicit cycles of exocytosis of the dye in the extracellular medium. After 30 minutes another fluorescent microscopic photo is taken, and neuronal activity is measured as the difference in fluorescence intensity before and after  $K^+$  stimulation.

Beta-amyloid is added to this fluorescent assay system in the form, and in the concentrations, described above. The added beta-amyloid protein inhibits vesicle cycling. The vesicle cycling correlates with neuronal long-term potentiation function. When GA and/or GJ, is added with the beta-amyloid protein in the concentrations described above, a reversal of beta-amyloid inhibition of vesicle cycling is seen. Derivative of GA, and/or GJ, as well as other structural analogs of GA and/or GJ, are rapidly screened for their ability to reverse beta-amyloid inhibition of vesicle cycling using this assay. Those with this activity are used in treating

neurodegenerative diseases or disorders as well as for the enhancement of cognitive function. Accordingly, this method allows the screening of multiple samples at the same time and, insomuch as it is not a substitute for electrophysiological recordings, it provides a fast and efficient tool to identify drugs, which affect neuron function.

### **Equivalents**

While this invention has been particularly shown and described with references to specific embodiments thereof, it will be understood by those skilled in the art that various changes in form and details can be made therein without departing from the spirit and scope of the invention as defined by the appended claims. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the appended claims.

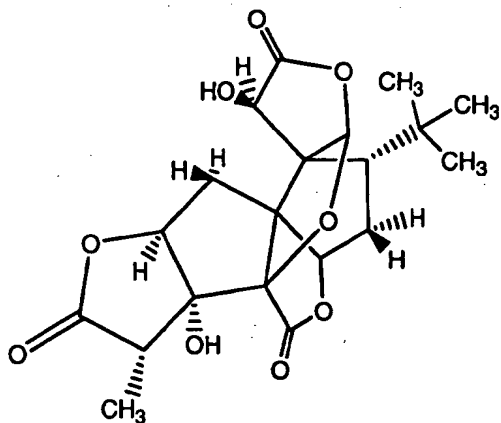


What is claimed is:

1. A composition consisting essentially of:

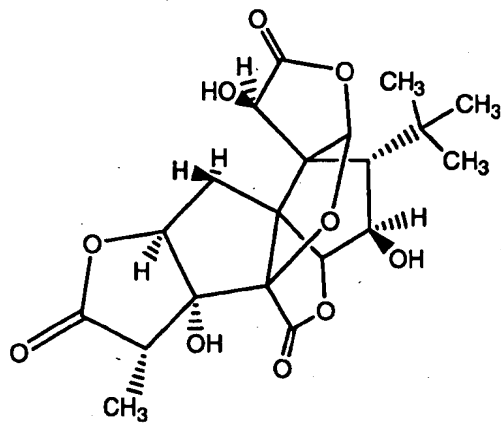
- (a) Ginkgolide A or a Ginkgolide A derivative; and
- (b) Ginkgolide J or a Ginkgolide J derivative.

2. The composition of claim 1, wherein the Ginkgolide A has the formula:



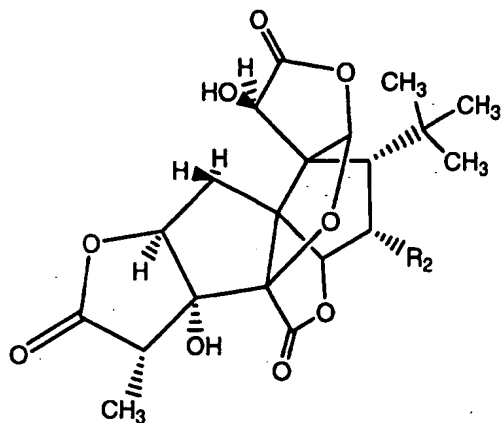
[2].

3. The composition of claim 1, wherein the Ginkgolide J has the formula:



[3].

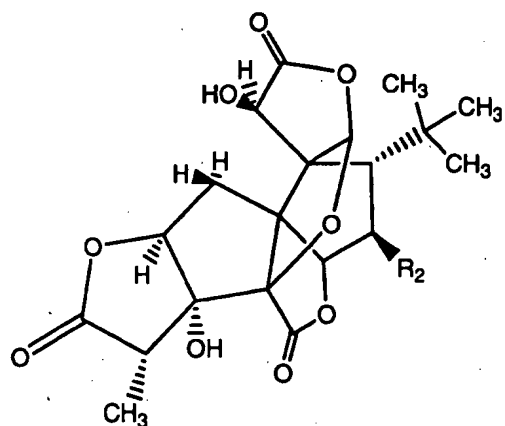
4. The composition of claim 1, wherein the Ginkgolide A derivative has the formula:



[5].

wherein  $R_2$  is  $NH_2$ , F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

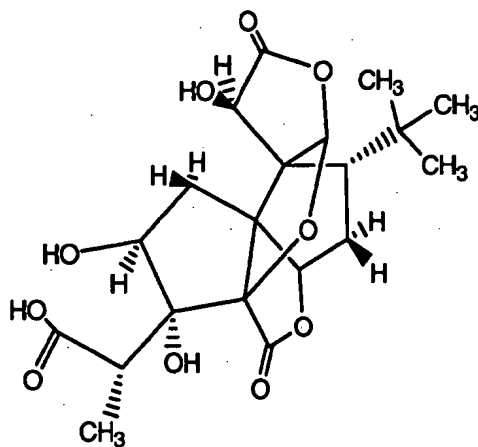
5. The composition of claim 1, wherein the Ginkgolide J derivative has the formula:



[4].

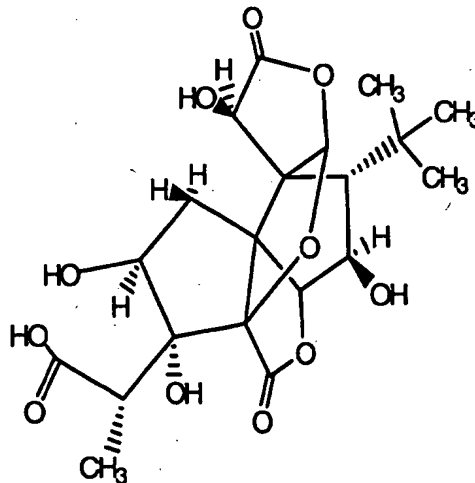
wherein  $R_2$  is  $NH_2$ , F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

6. The composition of claim 1, wherein the Ginkgolide A derivative is a monoacid Ginkgolide A derivative having the formula:



[6].

7. The composition of claim 1, wherein the Ginkgolide J derivative is a monoacid Ginkgolide J derivative having the formula:

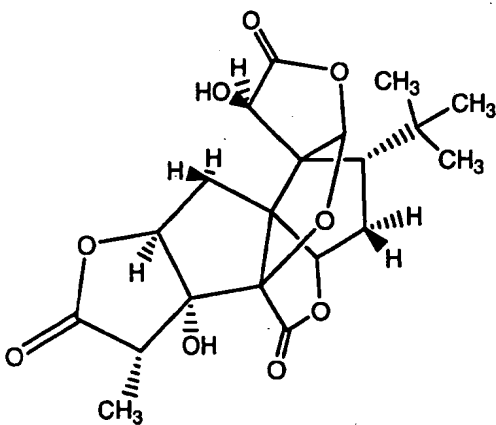


[7].

8. The composition of claim 1, consisting essentially of Ginkgolide A and Ginkgolide J.

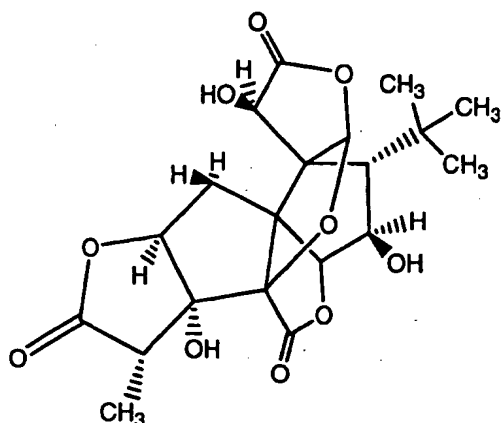
9. The composition of claim 1, consisting of Ginkgolide A and Ginkgolide J.

10. A composition consisting essentially of at least two compounds selected from the group consisting of Ginkgolide A, a Ginkgolide A derivative, Ginkgolide J and a Ginkgolide J derivative.
11. The composition of claim 10, wherein the two compounds are Ginkgolide A and a Ginkgolide A derivative.
12. The composition of claim 10, wherein the two compounds are both Ginkgolide A derivatives.
13. The composition of claim 10, wherein the two compounds are Ginkgolide J and a Ginkgolide J derivative.
14. The composition of claim 10, wherein the two compounds are both Ginkgolide J derivatives.
15. The composition of claim 10, wherein the Ginkgolide A has the formula:



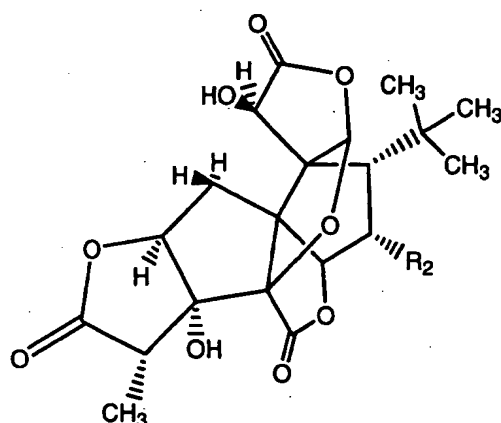
[2].

16. The composition of claim 10, wherein the Ginkgolide J has the formula:



[3].

17. The composition of claim 10, wherein the Ginkgolide A derivative has the formula:

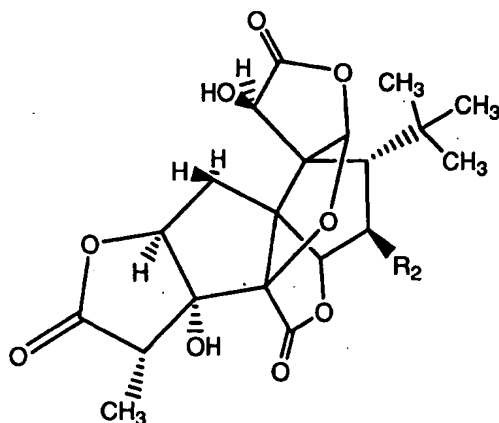


[5].

wherein R<sub>2</sub> is NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate

having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

18. The composition of claim 10, wherein the Ginkgolide J derivative has the formula:



[4].

wherein R<sub>2</sub> is NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

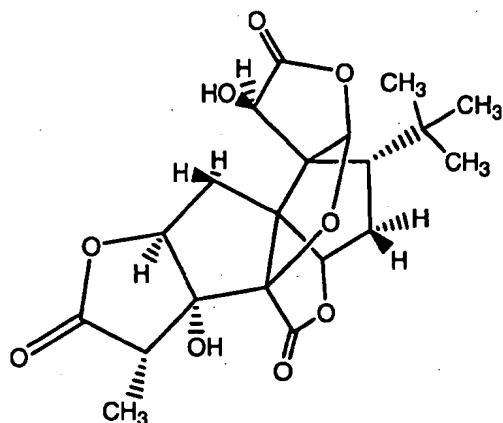
19. A composition consisting essentially of:

- (a) Ginkgolide A or a Ginkgolide A derivative;
- (b) Ginkgolide J or a Ginkgolide J derivative; and
- (c) a pharmaceutical carrier.

20. The composition of claim 19, wherein the pharmaceutical carrier comprises a carrier for oral administration, intravenous administration, administration via the respiratory tract, subcutaneous administration, intramuscular administration, rectal administration, topical administration or any combination thereof.

21. The composition of claim 19, wherein the pharmaceutical carrier comprises a sugar, a starch, cellulose, a cellulose derivative, malt, gelatin, a talc, an oil, a glycol, a glycerin, a polyethylene glycol, as ester, an aptamer, magnesium hydroxide, aluminum hydroxide, isotonic saline, Ringer's solution, ethyl alcohol, pyrogen-free water, phosphate buffered saline, sodium lauryl sulfate, magnesium stearate or any combination thereof.

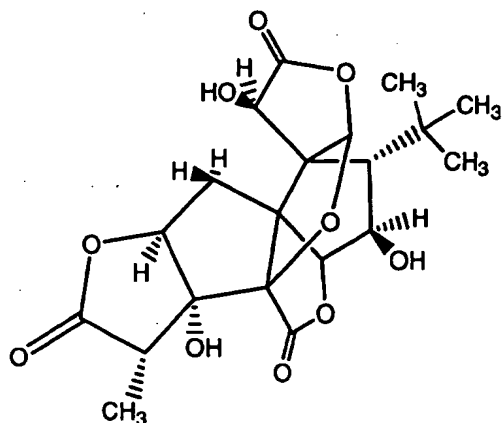
22. The composition of claim 19, wherein the Ginkgolide A has the formula:



[2].

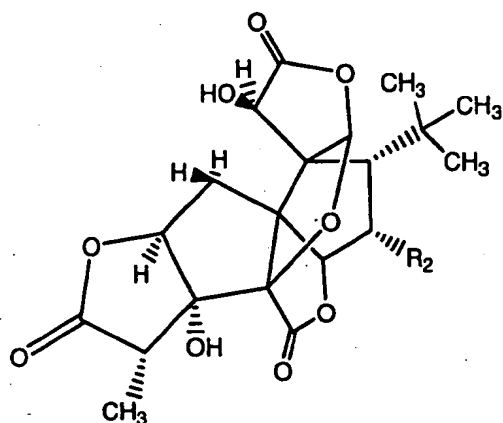
23. The composition of claim 19, wherein the Ginkgolide J has the formula:





[3].

24. The composition of claim 19, wherein the Ginkgolide A derivative has the formula:

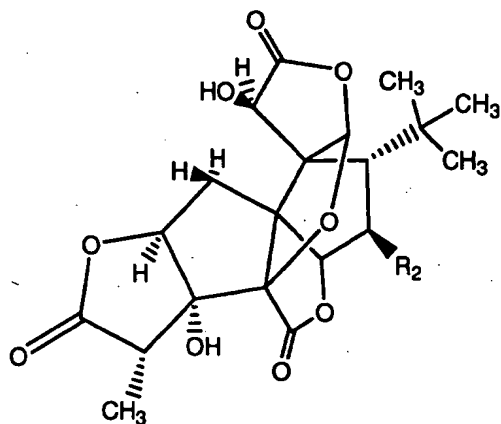


[5].

wherein  $R_2$  is  $NH_2$ , F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate

having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

25. The composition of claim 19, wherein the Ginkgolide J derivative has the formula:



[4].

wherein R<sub>2</sub> is NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

26. The composition of claim 19, consisting essentially of Ginkgolide A, Ginkgolide J, and a pharmaceutical carrier.

27. The composition of claim 19, consisting of Ginkgolide A, Ginkgolide J, and a pharmaceutical carrier.

28. A composition consisting essentially of:

- (a) Ginkgolide A or a Ginkgolide A derivative;
- (b) Ginkgolide J or a Ginkgolide J derivative; and
- (c) at least one antioxidant.

29. The composition of claim 28, consisting essentially of Ginkgolide A, Ginkgolide J, and at least one antioxidant.

30. The composition of claim 28, wherein the at least one antioxidant comprises a flavonoid.

31. The composition of claim 30, wherein the flavonoid comprises a flavonol glycoside.

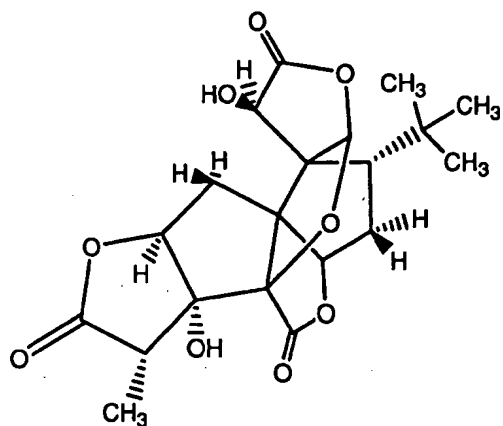
32. The composition of claim 31, wherein the flavonol glycoside comprises quercetin, kaempferol, isorhamnetin, or any combination thereof.

33. The composition of claim 28, wherein the at least one antioxidant comprises a biflavone.

34. The composition of claim 33, wherein the biflavone comprises amentoflavone, ailobetin, ginkgetin, isoginlgetin, sciadopirysin, or any combination thereof.

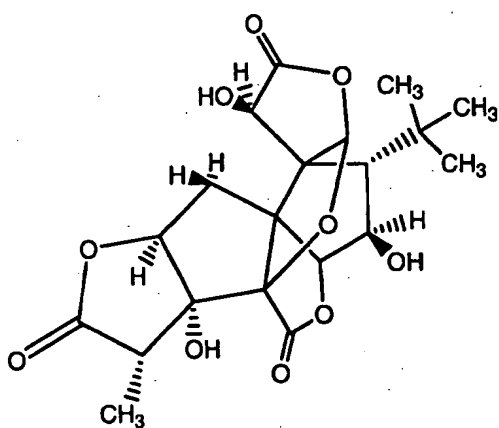
35. The composition of claim 28, wherein the antioxidant comprises vitamin C, vitamin E, N-acetyl-L-cysteine, resveratrol, coenzyme Q, alpha-lipoic acid, lycopene, or any combination thereof.

36. The composition of claim 28, wherein the Ginkgolide A has the formula:



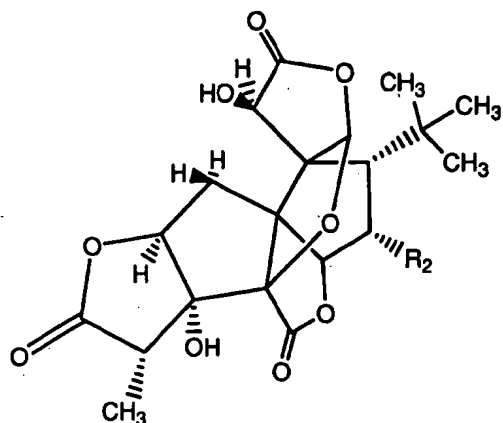
[2].

37. The composition of claim 28, wherein the Ginkgolide J has the formula:



[3].

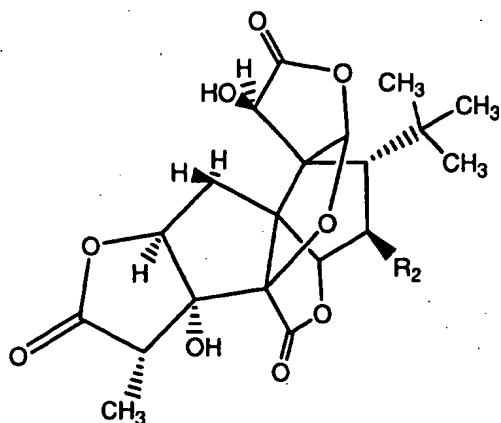
38. The composition of claim 28, wherein the Ginkgolide A derivative has the formula:



[5].

wherein  $R_2$  is  $NH_2$ , F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

39. The composition of claim 28, wherein the Ginkgolide J derivative has the formula:



[4].

wherein R<sub>2</sub> is NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

40. A composition consisting essentially of:

- (a) Ginkgolide A or a Ginkgolide A derivative;
- (b) Ginkgolide J or a Ginkgolide J derivative;
- (c) at least one flavonoid; and
- (d) a pharmaceutical carrier.

41. A composition consisting essentially of:

- (a) Ginkgolide A or a Ginkgolide A derivative;
- (b) Ginkgolide J or a Ginkgolide J derivative;
- (c) at least one antioxidant; and

(d) a pharmaceutical carrier.

42. A composition consisting essentially of Ginkgolide A, Ginkgolide J and bilobalide.

43. The composition of any of claims 1-18, 28-39, or 42, which further includes a pharmaceutical carrier.

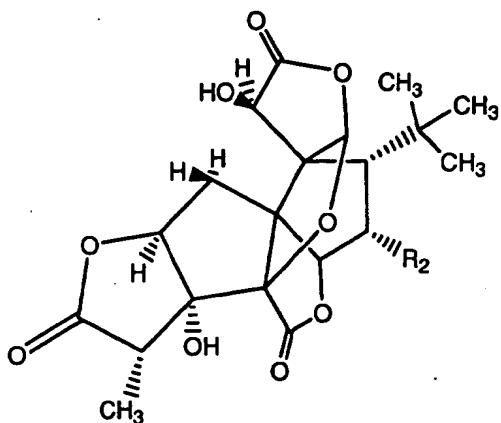
44. The composition of claim 43, wherein the pharmaceutical carrier comprises a carrier for oral administration, intravenous administration, administration via the respiratory tract, subcutaneous administration, intramuscular administration, rectal administration, or topical administration.

45. The composition of claim 43, wherein the pharmaceutical carrier is selected from the group consisting of a sugar, a starch, cellulose, a cellulose derivative, malt, gelatin, a talc, an oil, a glycol, a glycerin, a polyethylene glycol, as ester, an aptamer, magnesium hydroxide, aluminum hydroxide, isotonic saline, Ringer's solution, ethyl alcohol, pyrogen-free water, phosphate buffered saline, sodium lauryl sulfate, and magnesium stearate.

46. A composition comprising a Ginkgolide A derivative or a Ginkgolide J derivative.

47. The composition of claim 46, comprising a Ginkgolide A derivative.

48. The composition of claim 47, wherein the Ginkgolide A derivative has the formula:

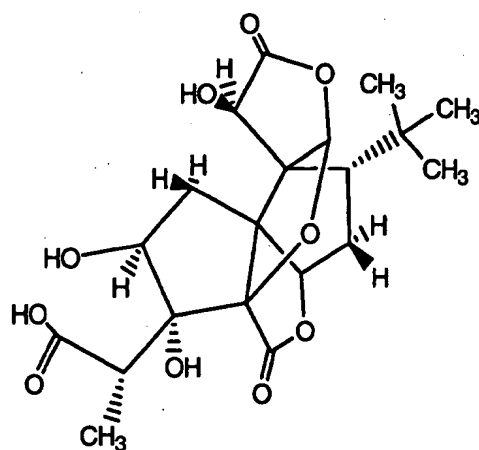


[5].

wherein  $R_2$  is  $NH_2$ , F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

49. The composition of claim 47, wherein the Ginkgolide A derivative is a monoacid Ginkgolide A derivative having the formula:

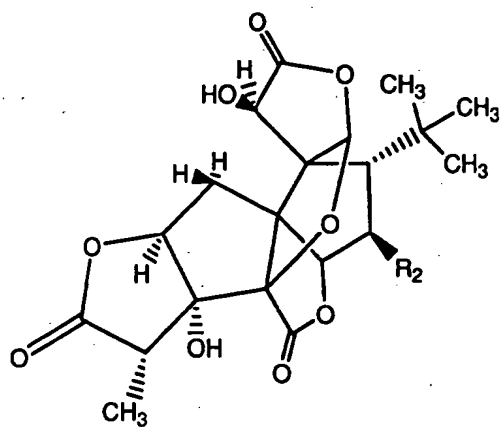




[6].

50. The composition of claim 46, comprising a Ginkgolide J derivative.

51. The composition of claim 50, wherein the Ginkgolide J derivative has the formula:

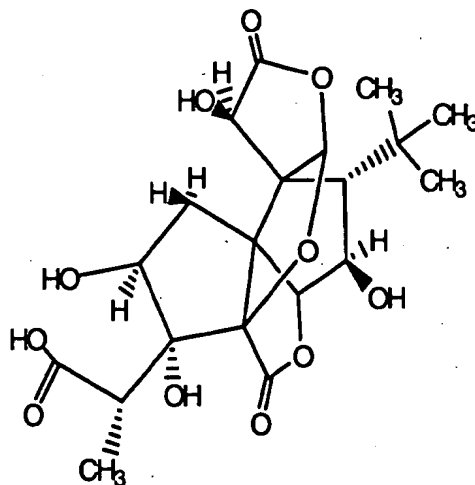


[4].

wherein R<sub>2</sub> is NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl

group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

52. The composition of claim 50, wherein the Ginkgolide J derivative is a monoacid Ginkgolide J derivative having the formula:



[7].

53. The composition of any of claims 1-42, or 44-52, wherein the composition is useful for treating a neurodegenerative disease or disorder.

54. The composition of any of claims 1-42, or 44-52, wherein the composition is useful for treating a memory disorder.

55. The composition of any of claims 1-42, or 44-52, wherein the composition is useful for improving memory.

56. The composition of claim 46, wherein the neurodegenerative disease or disorder is Alzheimer's disease.
57. The composition of claim 46, wherein the composition protects against neuronal cell death or long term potentiation impairment by beta amyloid protein.
58. The composition of claim 47, wherein the composition protects against neuronal cell death or long term potentiation impairment by beta amyloid protein.
59. The composition of claim 49, wherein the composition protects against neuronal cell death or long term potentiation impairment by beta amyloid protein.
60. A method of treating a neurological or neurodegenerative disease or disorder in a mammal comprising administering the composition of any of claims 1-42, or 44-52.
61. A method of treating a memory disorder in a mammal comprising administering the composition of any of claims 1-42, or 44-52.
62. A method of improving memory in a mammal comprising administering the composition of any of claims 1-42, or 44-52.
63. A method of treating depression in a mammal comprising administering the composition of any of claims 1-42, or 44-52.
64. The method of claim 60, wherein the neurological or neurodegenerative disease or disorder comprises Alzheimer's disease.
65. The method of claim 60, wherein the neurological or neurodegenerative disease or disorder comprises trisomy 21.

66. The method of claim 60, wherein the neurological or neurodegenerative disease or disorder comprises a neurodegenerative-related condition such as, but not limited to, multiple sclerosis, amyotrophic lateral sclerosis, Alpers' disease, corticobasal ganglionic degeneration, multiple system atrophy, motor neuron disease, olivopontocerebellar atrophy, Parkinson's disease, prion disease, Rett syndrome, tuberous sclerosis, Shy-Drager syndrome, Huntington's disease, senile dementia, epileptic dementia, presenile dementia, post-traumatic dementia, vascular dementia and post stroke dementia, alcoholism, meningitis, neonatal hypoxia, stroke, global cerebral ischemia, or any combination thereof.
67. The method of claim 60, wherein the composition promotes neurogenesis in the treated mammal.
68. The method of claim 60, wherein the composition is administered in an amount effective to protect against neuronal cell death or long term potentiation impairment by beta amyloid protein.
69. The method of claim 61, wherein the composition is administered in an amount effective to protect against neuronal cell death or long term potentiation impairment by beta amyloid protein.
70. The method of claim 64, wherein the composition is administered in an amount effective to protect against neuronal cell death or long term potentiation impairment by beta amyloid protein.
71. A method for treating neurological damage in a mammal comprising administering the composition of any of claims 1-42, or 44-52, in an amount effective to cause neurite out growth.
72. The method of claim 71, wherein the neurological damage comprises a spinal chord injury.
73. The method of claim 60, wherein the composition is administered orally, intravenously, via the respiratory tract, subcutaneously, intramuscularly, rectally, or topically.
74. A method for protecting a neuron against neuronal cell death or long term potentiation impairment by beta amyloid protein comprising contacting the neuron with Ginkgolide A.

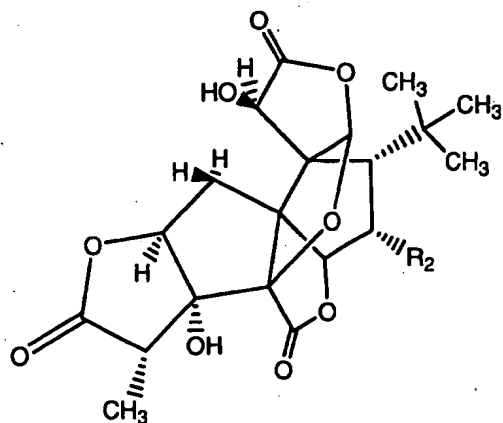
75. A method for protecting a neuron against neuronal cell death or long term potentiation impairment by beta amyloid protein comprising contacting the neuron with Ginkgolide J.

76. A method for protecting a neuron against neuronal cell death or long term potentiation impairment by beta amyloid protein comprising contacting the neuron with Ginkgolide A and Ginkgolide J.

77. The method of claim 74, wherein the neuron is contacted with a composition consisting essentially of Ginkgolide A and Ginkgolide J.

78. A method for stimulating axonal out growth of a neuron comprising contacting the neuron with Ginkgolide A.

79. A method for stimulating axonal out growth in a neuron comprising contacting the neuron with a Ginkgolide A derivative having the formula:



[5].

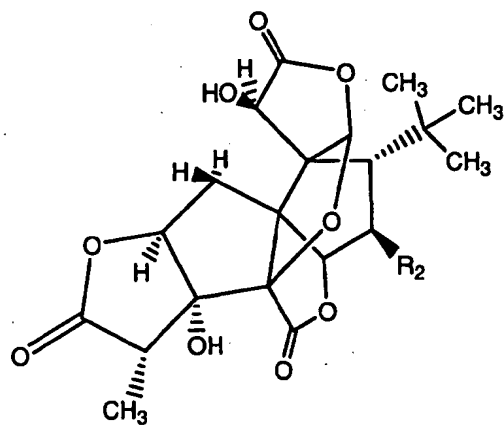
wherein  $R_2$  is  $NH_2$ , F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl

group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

80. The method of claim 78, further comprising contacting the neuron with Ginkgolide J or a Ginkgolide J derivative.

81. A method for protecting a neuron from neuronal cell death comprising contacting the neuron with Ginkgolide J.

82. A method for protecting a neuron from neuronal cell death comprising contacting the neuron with a Ginkgolide J derivative having the formula:



[4].

wherein R<sub>2</sub> is NH<sub>2</sub>, F, Cl, Br, I, an unsubstituted or substituted straight or branched alkyl group having 1 to 5 carbon atoms, an alkenyl group having 1 to 5 carbon atoms, an alkynyl group having 1 to 5 carbon atoms, an O-alkyl having unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-alkenyl group with 1 to 5 carbon atoms, an O-alkynyl group with 1 to 5 carbon atoms, an O-CO-alkyl ester having an unsubstituted or substituted

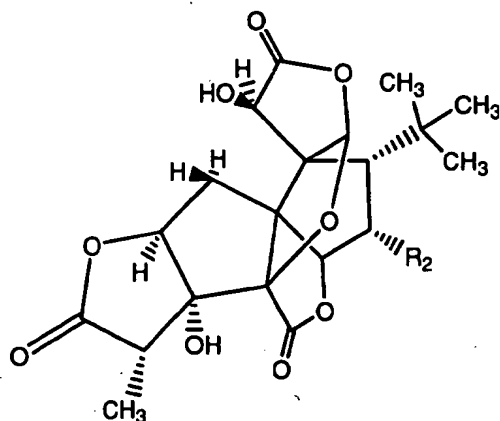
straight or branched alkyl group with 1 to 5 carbon atoms, an O-CO-aryl ester that can be substituted or unsubstituted, an O-CO-NH-alkyl carbamate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, an O-SO<sub>2</sub>-alkyl sulfonate having an unsubstituted or substituted straight or branched alkyl group with 1 to 5 carbon atoms, or an O-SO<sub>2</sub>-aryl sulfonate that can be substituted or unsubstituted.

83. The method of claim 81, further comprising contacting the neuron with Ginkgolide A or a Ginkgolide A derivative.

84. A method of treating a neurological or neurodegenerative disease or disorder in a mammal comprising administering an enriched *Ginkgo biloba* extract comprising at least about 60% terpene trilactones.

85. A method of protecting a neuron against neuronal cell death or long term potentiation impairment by beta amyloid protein comprising contacting the neuron with an enriched *Ginkgo biloba* extract comprising at least about 60% terpene trilactones.

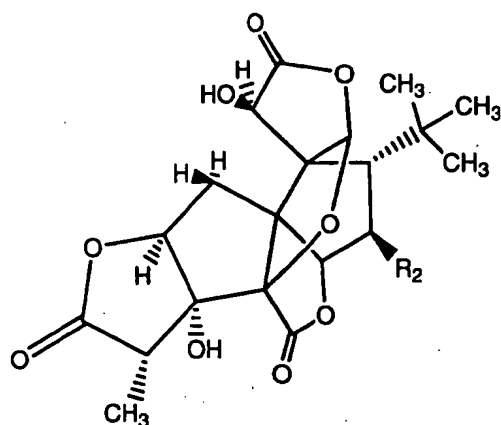
86. A method of identifying a receptor that binds a compound that protects against neuronal cell death or long term potentiation impairment by beta amyloid protein comprising administering a Ginkgolide A derivative having the formula:



[5],

wherein  $R_2$  is a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety.

87. A method of identifying a receptor that binds a compound that protects against neuronal cell death or long term potentiation impairment by beta amyloid protein comprising administering a Ginkgolide J derivative having the formula:



[4],

wherein  $R_2$  is a photoactivatable moiety, a fluorescent moiety, or a radioactive moiety.

88. The method of claim 86 or 87, wherein the method is diagnostic of Alzheimer's disease.



**ABSTRACT**

The invention provides ginkgolide-based compositions, including Ginkgolides A and/or J, and/or derivatives thereof, and associated methods for the treatment or prevention of neurological or neurodegenerative disease associated with neuron cell death and/or inhibition of neuronal long-term potentiation including Alzheimer's disease, Down's syndrome, multiple sclerosis, amyotrophic lateral sclerosis, motor neuron disease, Parkinson's disease, prion disease, Huntington's disease, as well as, depression, head trauma, stroke, and CNS hypoxia.

**Figure 1**

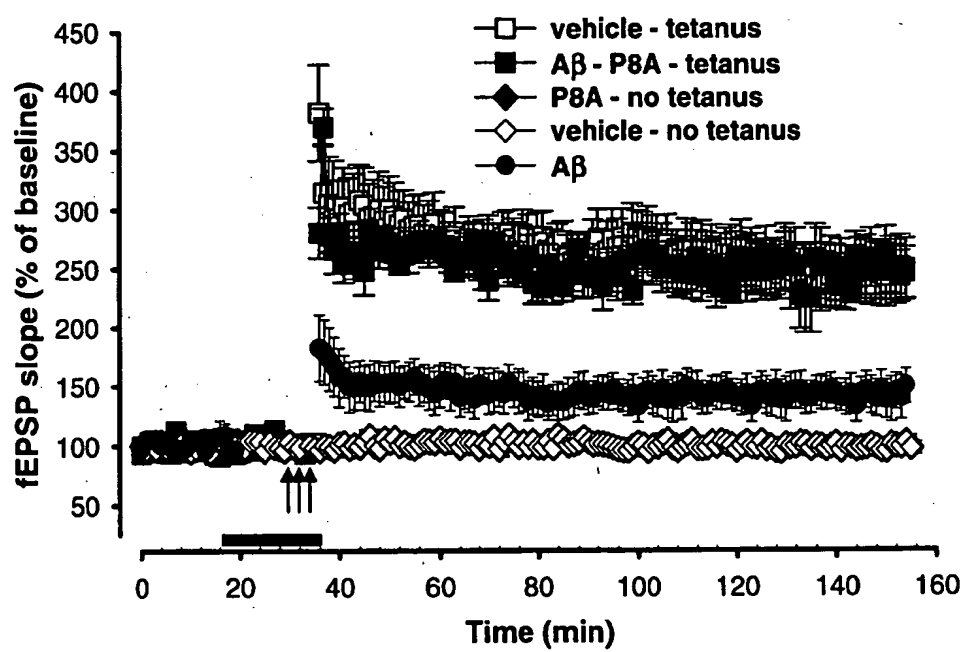
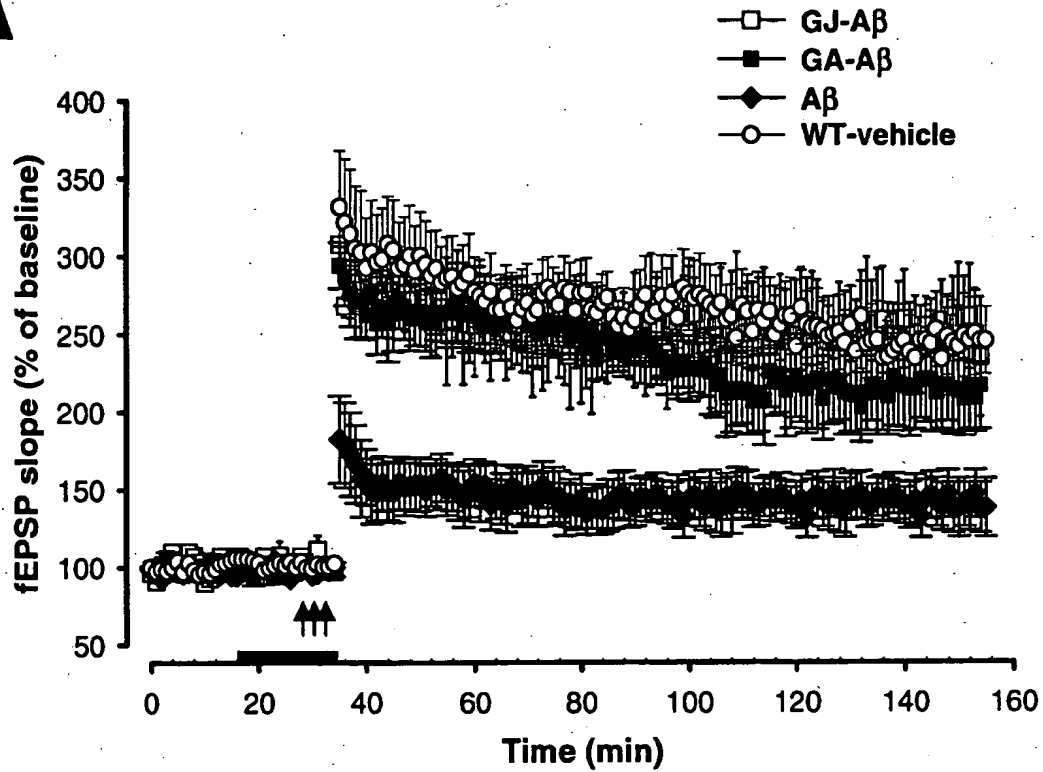
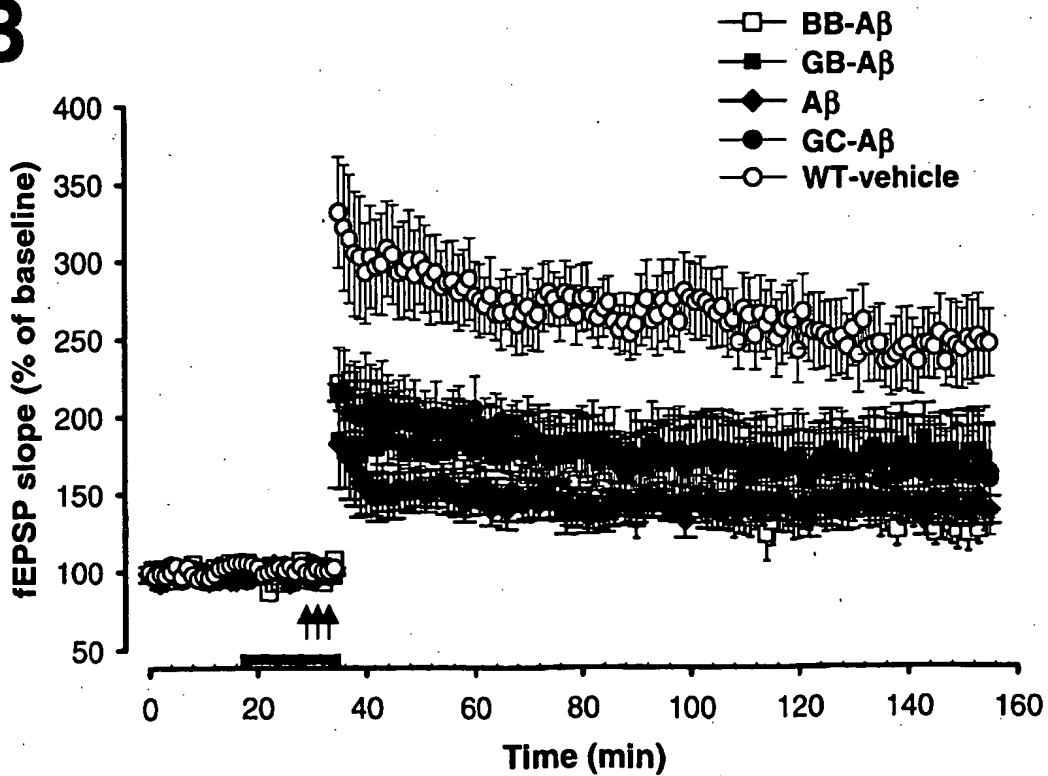


Figure 2

**A**



**B**



**Figure 3**

